

DIAGNOSTIC VALUE OF GENE PROBES AND ITS CORRELATION WITH CLINICAL PROFILE OF LEPROSY IN CHILDREN

R. Dayal
P.K. Agarwal
K. Kalra
V.P. Bharadwaj
V.M. Katoch
K. Katoch

ABSTRACT

Clinico-bacteriological profile of 73 leprosy patients below 16 years of age was studied. Majority of the patients were males and fell in 11-16 years age group ($p < 0.05$). Skin lesions were present in all cases on both exposed as well as unexposed areas and their number increased with advancing age. Cutaneous sensations were affected in most of the patients while nerve thickening was observed in 41. As age increased, the disease moved from the tuberculoid end of spectrum towards the lepromatous end ($p < 0.05$) and the positivity of the skin smears increased ($p < 0.05$). Majority of the paucibacillary cases were lepromin positive while most multibacillary cases were lepromin negative ($p < 0.01$).

*Two *M. leprae* specific gene probes were applied in 42 cases to assess their diagnostic value. Eighty one per cent cases were picked up by the probes indicating presence of active bacilli. These included all lepromin positive cases, all smear positive cases, and most of smear negative cases ($p < 0.05$). Seven children with inconclusive histology were also positive. Drug treatment and inadequate size of biopsy sample could explain the negative probe results in 19% cases.*

This study highlights the immense potential of gene probes in diagnosing leprosy in children.

Key words: Gene probes, Leprosy.

There are an estimated 12 million leprosy sufferers throughout the world(1). In India the figure is considered to be around 4 million, of whom about one quarter are below the age of 16 years. In the city of Agra, the prevalence rate is 1.4/1000(2).

Children with overt disease form the tip of the iceberg. For eradication of disease, early diagnosis is essential. At present conventional methods of diagnosis include clinical and histopathological features, serological tests like Fluorescent Leprosy antibody-absorption technique (FLA-ABS), various ELISAs and specific radiometric competition immunoassays. These tests are relatively less sensitive in smear negative paucibacillary cases, and clinically atypical cases with non-specific histopathology and non-detectable acid fast bacilli(3,4). Such cases need alternative objective tests for confirmation. The application of recently developed gene probes and/or other gene amplification systems which are highly sensitive and specific, thus assumes great importance.

With the advances made in gene probe technology, probes and gene amplification systems for *M. leprae* are now becoming available(3,4). However, hardly any studies are available in the Indian literature on their application in childhood leprosy. With this background, the present study was performed:

To detect nucleic acids of *M. leprae* in skin lesions of cases clinically suspected

From the Department of Pediatrics, S.N. Medical College, Agra and Central JALMA Institute of Leprosy, Agra.

Reprint requests: Dr. R. Dayal, Opposite Kidwai Park, Rajamandi, Agra 282 003.

Received for publication: July 8, 1994;

Accepted: September 2, 1994

of having leprosy, by gene probes.

2. To study the clinical profile of leprosy in children and assess its correlation with the findings of gene probes.

Material and Methods

This study was conducted at the Department of Pediatrics, S.N. Medical College and Hospital, Agra and Central JALMA Institute for Leprosy, Agra. Seventy three clinically diagnosed cases of leprosy below 16 years of age, who attended the Out-Door Patient Department were studied. Forty healthy contacts were taken as controls.

All patients were subjected to a detailed history and thorough clinical examination. Most of them had smear examination and lepromin test done. Skin smears, taken from earlobes and the site of lesion were stained by Ziehl-Neelsen staining and examined under oil immersion lens (100X). Skin biopsy was taken from all controls and skin lesions of 42 patients and subjected to histopathological examination and gene detection.

Nucleic acids were isolated by the standard procedure of Katoch *et al*(5) from the tissue homogenate prepared from skin biopsies. This r-RNA was purified by standard technique.

Gene probes were synthesized in a Pharmacia Gene Assembler Plus by the procedure detailed in the manual. Oligonucleotide probes used in this study were:

1. 17-mer synthetic DNA Oligonucleotide probe 5' CACT GGCT TCGG GTGT T 3' against a strength of 1425-1441 positions (P₁)(6).
2. 18 per oligonucleotide probe: CTTC AAGG CGGA TGTC TT targeting 192-209 regions of 16S-r-RNA of *M. leprae* (P₂)(7).

After radiolabelling with r-p³² ATP(8), these probes were purified in G-50 sephadex column. Purified nucleic acids (r-RNA) were blotted on nitrocellulose membrane using dot blot apparatus (USA) with vacuum pump. Hybridization with probes and washing of hybridized membrane was done under the condition described by Katoch *et al.* (6,7). After hybridization, the membrane was subjected to autoradiography at — 70°C. The results were interpreted after getting the X-ray films processed and compared with background signals from controls.

Results

A total of 73 cases of less than 16 years of age were studied. Majority of them (80%) were males. Maximum number of cases (74%) fell in the 11-16 years age group (p <0.05). As the age advanced, the disease moved from the tuberculoid end of the spectrum towards the lepromatous end. This finding was statistically significant (p <0.05) (Table I).

As the disease moved towards the lepromatous end of the spectrum, the number of skin lesions increased. Majority of patients had macular (69.86%) and hypopigmented lesions (68.49%) lesions, present over both covered as well as uncovered body areas. Cutaneous sensations were affected in most of the patients while nerve thickening was observed in 41. Skin smear examination was done in 71 cases; of these majority of paucibacillary cases (93%) were smear negative while most of multibacillary cases were smear positive (66.7%).

Lepromin test was done in 42 patients. It was positive in 52% patients. All cases with indeterminate(I) and tuberculoid (TT) lesions and majority of borderline tuberculoid (BT) patients give a positive re-

TABLE III—Correlation of Smear Status with Results of Probe Tests

Type of disease	No. of cases	Smear status	No. of cases	Results of probe tests			
				Probe 1		Probe 2	
				+	-	+	-
PB	I	+	—	—	—	—	—
		-	2	1	1	—	2
	BT	+	2	1	1	1	1
		-	15	11	4	8	7
MB	BB	+	10	10	—	8	2
		-	6	3	3	3	3
	BL	+	2	2	—	2	—
		-	3	2	1	2	1
	LL	+	2	2	—	2	—
		-	—	—	—	—	—
Total	42	42	32	10	26	16	

PB— $sP_1 = -0.688$, $p < 0.05$, $sP_2 = -0.229$, $p > 0.05$, $P_1P_2 = +0.192$, $p > 0.05$.

MB— $sP_1 = +0.692$, $p < 0.05$, $sP_2 = +0.466$, $p < 0.05$, $P_1P_2 = +0.626$, $p < 0.05$.

of P_1 was statistically significant ($p < 0.05$). Among 16 negative reactors, both probes detected 10 common cases and each probe detected one extra case. Here also the correlation with both the probes was statistically significant ($p < 0.05$) (Table IV).

Histology was done in 17 out of 42 probe tested cases. Out of 4 cases showing histology of indeterminate (I) type, 2 cases were detected by P_1 and one case by P_2 . All the 3 cases with a non-specific histology were positive by both the probes. In TT, BT, BB, BL and LL groups, all cases were detected by one or both the probes (Table V).

Discussion

Very few studies are available in the Indian literature on childhood leprosy, particularly on the value of gene probes as a diagnostic tool.

The incidence of the disease increased significantly as the age advanced, being highest in the 11-16 years age group. This is in parallel with other studies(9-12) and is possible because of the long incubation period of the disease. On the other hand, scanty reports of the disease occurring in infancy are also available(13-15). In these rare instances, *in utero* transmission of the

end of the spectrum, the smear positivity increased.

The significantly low positivity of the lepromin test in children towards the lepromatous end of the spectrum demonstrates their poor cellular immune response. This has been reported by other researchers also(21). In this series only the late (Mitsuda) response to lepromin was noted as that is regarded to be a more reliable index of cell mediated immunity(22). As far as childhood patients of leprosy are concerned, the main value of lepromin test lies in confirming the results of classification of cases based on clinical and bacteriological grounds, and in assessing the prognosis as a positive lepromin test signifies a good cellular immune response.

It was observed that cases with a positive lepromin test were mostly bacteriologically negative while cases with a negative lepromin test were mostly smear positive.

Clinico-histological correlation was observed only in 6 out of 18 cases. In 12 cases, upgrading of disease was seen. This again could be due to the treatment received.

Gene probes have been used in the diagnosis of many disorders, *e.g.*, thalassemia, muscular dystrophy, *etc.* but very few studies are available on their application in the diagnosis of childhood leprosy. In our pioneer study two probes designed in India(6,7) were applied in 42 cases. Twenty four cases detected were common to both the probes. Probe 1 and probe 2 confirmed the diagnosis in 8 and 2 extra cases, respectively. All smear positive cases and majority of smear negative were picked up by one or both the probes. The value of the probes is further emphasized by the fact that they picked up disease in 7 children whose histopathology was inconclusive (4-indeterminate, 3-non-

specific). Again, all lepromin positive cases were picked up by the probes. The overall performance of probe 1 was better than probe 2.

Seven out of a total of 42 cases, who were not picked up by the probes, were smear negative. Possible explanations for this observation are: (a) these cases were on treatment which reduced the bacilli load; and (b) inadequacy of biopsy sample.

This study establishes that gene probes are a valuable diagnostic tool for childhood leprosy particularly for cases who are paucibacillary and smear negative. As the positivity is nearly 73% in smear negative cases, amplification is generally not required. However, to further evaluate those children who are negative by probes but have clinical evidence of disease, polymerase chain reaction may be used to amplify the sensitivity of the probe. For smear positive cases, these probes can be used for objective confirmation of diagnosis.

To conclude, this study proves the immense potential of gene probes in the diagnosis of leprosy. As this study involved a small number of cases, it would be advisable to launch larger surveys to further establish the applicability and value of probes in early diagnosis.

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