Lepr Rev (1990) **61,** 145–150

Serodiagnosis of leprosy in patients' contacts by enzyme-linked immunosorbent assay

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Accepted for publication 10 November 1989

Summary Serum samples from 3336 contacts of leprosy patients were tested for antiphenolic glycolipid I antibodies by enzyme-linked immunosorbent assay with the albumin coupled synthetic disaccharide antigen. The overall positivity rate was 9·3%. No significant differences were seen between a group of household contacts of lepromatous patients and those of the other types of the disease. The proportion of ELISA positives was slightly higher in the relatives as compared to workplace contacts and neighbours but significantly different only between the two former ($p < 0{\cdot}05$). Among those contacts with absorbance values higher than 0·100, 5 new leprosy patients were diagnosed, 2 of them with positive skin smears. A sixth contact was detected with a very high absorbance value in whom no single skin lesion was found but whose lepromin reaction was 0 mm and the skin smear showed a bacteriological index of 3+.

Introduction

It has been suggested that since modern chemotherapy rapidly reduces the number of viable *Mycobacterium leprae* spread by an individual, the major source of leprosy transmission is likely to be individuals who do not yet have symptoms.[1] One of the principal goals of leprosy research is to develop tests that will allow identification and early treatment of such individuals, aimed at reducing transmission of the disease.[1] The incorporation of early diagnosis, especially of multibacillary cases, to the activities of integrated control programmes through appropriate methods of case finding is among the specific approaches considered by WHO to prevent and control leprosy.[2]

However, early diagnosis may not always be easy to attain. Symptoms of leprosy are very often absent or minimal, particularly during the early stages of the disease, hence patients do not voluntarily seek relief through the health care system,[3] whereas, in other instances, individuals in whom the disease has already manifested do not look for medical care either because of the social stigma or lack of health education. On the other hand, contact surveillance identifies only a small proportion of the total number of new cases

0305-7518/90/061145+06 $01.00

occurring in a community[3] and frequently in low endemic areas leprosy is not borne in mind, thus resulting in late diagnosis.

The isolation and chemical characterization of a *M. leprae* species specific antigen, the phenolic glycolipid I (PGI)[4,5] as well as the preparation of an artificial antigen containing the species specific moiety of PGI[6,7] have made the detection and study of anti-*M. leprae* specific antibodies possible by rather simple serological methods. It has been suggested that these antigens have considerable potential as tools in the serodiagnosis of subclinical stages of leprosy infection, early diagnosis of clinical disease and in detecting transmitters of infection.[8–10]

In this report data are presented from a trial based on the enzyme-linked immunosorbent assay (ELISA) using the albumin coupled synthetic disaccharide (ND-A-BSA) antigen to study anti-PGI antibody levels among contacts of leprosy patients and to seek for subclinical transmitters, cases with early clinical stages and individuals at risk of contracting the disease among those presumably infected.

Materials and methods

SERA

Serum samples from 3336 contacts were tested, of these, 660 were from household contacts, grouped according to the leprosy type (Madrid classification) of the index case, 2134 were relatives in whom no separation into household and non-household or leprosy type of the index case was made, 435 were workplace contacts and 107 were neighbours. They were all living in the province of Guantanamo, Cuba, where leprosy is highly endemic. Venous blood samples were taken, sera obtained and kept frozen at $-20°C$ until used.

ELISA

The procedure was the same as that described by Cho *et al.*[11] except that the antigen was not sonicated but dissolved directly in the buffer and added to wells of flexible PVC plates (Flow Laboratories). The ND-A-BSA antigen was obtained from IMMLEP (Gigg DISSACH). The conjugate reagent was goat anti-human IgM-peroxidase conjugated IgG fraction (Cappel Laboratories). The absorbance was read at 492 nm in a Titertek Multiskan MC or in an Organon Technika reader. A serum was considered 'positive' when the absorbance exceeded 0·160 since this value corresponded to the 98th percentile when 100 serum samples obtained from a Havana blood bank were tested to establish the cut-off level.

Dermatological and skin-smear examinations were performed in contacts who showed absorbance values above 0·100 and not in those with lower values. Mitsuda tests with lepromin A, obtained from G. W. Long Hansen's Disease Center, LA 70721, USA, were also included in the clinical study of 200 of such contacts.

Differences between proportions above the cut-off level among the groups of contacts were ascertained by the hypotheses test for two proportions and correlation was also done between the results of ELISA and Mitsuda tests.

Results

The overall ELISA positivity rate of all contacts tested was 9·3%. Table 1 shows the test results for household contacts grouped according to the leprosy type (Madrid classification) of the index case. No significant differences were seen between the contacts of lepromatous patients and those of the other types of the disease.

The data for other relatives (household and non-household), workplace contacts and neighbours of all leprosy patients are shown in Table 2. The proportion of ELISA positives was slightly higher in the relatives as compared to workplace contacts and neighbours but significantly different only between the two former ($p < 0{\cdot}05$).

The observed distribution of absorbance values is shown in Table 3. Of the total, 81·7% were below 0·100, 12·4% were in the range 0·101–0·200, 3·3% in the range 0·201–0·300 and 2·3% above 0·300. Hypochromic macules found in 2 (0·5%) of 417 contacts, in the range 0·101–0·200, hypoaesthetic in 1 of them, led to the diagnosis of indeterminate leprosy (patients 1 and 2). Hypochromic macules and a positive Fite–Faraco stained histological section found in 1 (4·7%) of 21 contacts in the range of 0·401–0·500 led to the diagnosis of the third indeterminate patient. In 1 (50·0%) of 2 in the range 0·701–0·800 clinical signs of lepromatous leprosy and a bacteriological index (BI) of 4+ were found (patient 4). Two contacts were found with absorbance values above 1·000, one of them with skin lesions and a BI of 2+ was diagnosed as a dimorphous leprosy whereas in the other one not a single skin lesion or other dermatological sign was found but the skin smear revealed a BI of 3+ (patients 5 and 6) Tables 3 and 4.

Mitsuda tests were performed in 200 contacts who showed an absorbance value higher than 0·100. The correlation coefficient (r) was $-0{\cdot}18630$ which is out of the $+/-$ 0·13808 critical interval for a $p = 0{\cdot}05$, thus a significant inverse correlation was proved between the lepromin reaction sizes and the absorbance values observed.

Table 1. ELISA results in household contacts grouped according to the leprosy type of the index case

Leprosy type of the index case*	Positive/Total	%
Lepromatous	38/350	10·8
Dimorphous	12/144	8·3
Indeterminate	10/123	8·1
Tuberculoid	5/43	11·6

* Madrid classification.

Table 2. ELISA results in other contacts of leprosy patients

Type of contact	Positive/Total	%
Relatives (household and non-household)	216/2134	10·1
Work place contacts	23/435	5·2
Neighbours	8/107	7·4

Table 3. Results of the serological tests, number of skin-smears performed and new cases detected

Absorbance level	Sera	Skin smears	Cases
<0·100	2728 (81·7)*	NE	NE
0·101–0·200	417 (12·4)	213 (51·0)†	2 (0·5)†
0·201–0·300	111 (3·3)	66 (59·4)	0
0·301–0·400	45 (1·3)	29 (64·4)	0
0·401–0·500	21 (0·6)	13 (61·9)	1 (4·7)
0·501–0·600	7 (0·2)	5 (71·4)	0
0·601–0·700	3 (0·08)	3 (100·0)	0
0·701–0·800	2 (0·08)	2 (100·0)	1 (50·0)
0·801–0·900	0	0	0
0·901–1·000	0	0	0
>1·000	2 (0·05)	2 (100·0)	2 (100·0)

NE, not examined.
*, percentage of the total number in brackets.
†, percentage of the number in the range in brackets.

Table 4. New leprosy cases diagnosed among contacts of leprosy patients

Patient	Leprosy type*	Mitsuda (mm)	ELISA	BI
1	Indeterminate	0	0·109	0
2	Indeterminate	3	0·125	0
3	Indeterminate	5	0·401	0
4	Lepromatous	0	0·755	4+
5	Dimorphous	0	2·125	2+
6	Subclinical infection	0	1·074	3+

* Madrid classification.

Discussion

The ELISA technique with the PGI or its semisynthetic analogues appears to be the most widely used system for the detection of anti-*M. leprae* specific antibodies at present. However not many reports on studies with high risk groups have been published so far. The overall serological positivity rate observed in this study (9·3%) was lower than that found by other workers in Mexico (23%) and Sri Lanka (33%),[12] but had the same definition of positivity ($A_{492} > 0·09$) been used by us it would have been comparable. It was close to those found by Douglas *et al.* (11·2%) in Cebu, Philippines[13] and by Chanteau *et al.* (12·8%) in French Polynesia.[14]

Contrary to what was expected, no significant differences were found between the proportions of positive household contacts grouped according to the leprosy type of the index case. It is likely that the contacts of paucibacillary patients may have been exposed

to the same infection sources of their index cases or to others in the community since they were living in a neighbourhood with a high prevalence rate (5·6 per 1000). Seropositivity in the relatives was higher than in the workplace contacts and neighbours but not statistically significant with respect to the latter. The fact that the group of neighbours was represented by persons living on the same block, where as many as 16 patients also lived, might account for this observation since it can be supposed that the risk of infection was high with so many patients in the vicinity.

Previous work with several immunological tests revealed that infection by *M. leprae* is much more frequent than the number of manifest clinical cases would indicate.[10] While the only accurate way of determining whether the test is useful as a serodiagnostic tool would be to examine clinically and bacteriologically all contacts. In the present study only those with absorbance values above 0·100 were chosen to be studied. The reason was that the work was aimed mainly at assessing the usefulness of the system, by testing serum samples from a rather large number of contacts, for detecting transmitters of infection who might be individuals who did not yet have physical signs and/or cases in very early stages of clinical manifestation, as well as for identifying those presumably infected who might be suspected of incubating multibacillary leprosy and, hence, might be potential transmitters. On the basis of accumulated data, it was assumed that subsequent multiplication of *M. leprae* after infection in such individuals would reach such a bacillary load that it would be reflected in absorbance values higher than 0·100.

In this study the majority of the positive individuals showed low absorbance values which might reflect normal immune responses to *M. leprae* exposures, a few false positive reactions as has been reported[6] and, in some cases, early stages of infection which could progress towards clinical disease. Much caution should be taken with the small number of contacts exhibiting high absorbance values since they are generally associated with multibacillary leprosy. In this connection the finding of a subclinically infected contact with acid-fast bacilli in his skin smears is of great interest, since this lends support to the assumption that these individuals may play an important role in the transmission of the disease.

It has been reported that this assay is not able to detect a high proportion of paucibacillary patients without considerable loss of specificity.[15] However, the usefulness of the system as a test for leprosy infection and its use for control purposes should be discussed in relation to particular epidemiological situations and to the quality of the control programme. There is evidence that the distribution of the different types of the disease may vary between different populations.[16] In Cuba, the proportion of multibacillary leprosy (lepromatous and dimorphous in the Madrid classification) reaches about 50% of the annually detected cases.[17] Therefore, in areas with a relatively high prevalence of multibacillary leprosy, the value of this tool for screening purposes may be important in terms of leading to the detection of a number of early cases and the identification of presumably infected individuals who could be examined and followed up. In addition, the administration of chemoprophylaxis to these latter subjects could also be evaluated.

Acknowledgments

This investigation received financial support from the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases.

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