Assessment of subclinical leprosy infection through the measurement of PGL-1 antibody levels in residents of a former leprosy colony in Thailand

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Introduction

Leprosy control in Thailand has been integrated into the general health facilities since 1973. Multidrug Therapy (MDT) was introduced in 1984 and gradually expanded throughout the whole country. This has resulted in a dramatic decrease in prevalence and incidence. As of 31 December 1994, the registered prevalence was just below 1 per 10 000, and it had reached a level of 0·21 per 10 000 by the end of 2006. Leprosy is unevenly distributed in the country. High prevalence rates are found in one province and 34 districts.

There are 13 leprosy colonies in Thailand, which can accommodate about 4500 ex-patients. Phra-Pradaeng Colony, which is affiliated with Raj Pracha Samasai Institute in Samutprakarn Province, is a suburban community in close proximity to Bangkok, the capital.
of Thailand. It is a congested community of 1000 ex-patients and about 2500 household members, residing within an area of 0.128 sq. km. The community has had an MDT-based leprosy control programme in operation for 20 years. During 2002–2004 there had been 1–3 relapsed leprosy cases detected annually in the community, all having completed DDS monotherapy more than 20 years ago. Those partially treated patients might have been the source of active transmission of leprosy bacilli in the community.

This sero-epidemiological study on contact transmission in leprosy was designed to investigate the hypothesis that close contacts of leprosy patients may already have been infected by \textit{M. leprae} by the time the patient is recognised and treatment started. Hence, Phra-Pradaeng Colony was selected for the study to test and offer the potential to conduct community-based intervention studies.

**Materials and Methods**

Household contacts and ex-patients in Phra-Pradaeng Colony were visited by the investigators from house to house. All household contacts and ex-patients in the colony have been assumed to be equally exposed to \textit{M. leprae} infection, because this tiny, congested area is highly endemic with a prevalence of about 10 per 10,000 population. Owing to the limitation of the research budget, only the first 400 household contacts and ex-patients encountered would be enrolled. The basis of the study was then explained, the proposed methods described, and agreement for the participation was secured. For children, consent from a guardian was needed. Data on the demographic characteristics and history of anti-leprosy treatments were collated. Each individual was clinically examined for signs of active leprosy.

If leprosy was diagnosed, the newly found patient was referred to the leprosy clinic for appropriate treatment and was also excluded from the study.

Exclusion criteria for contacts were as follows:

- Any person who refused informed consent.
- Any person resided temporarily in the area.

Blood was collected from individuals by venipuncture, and then left to clot. The serum was separated by centrifugation and stored at $-30^\circ$C until analysed by the ML Flow.

The ML Flow is a simple assay for the detection of IgM antibodies to \textit{M. leprae} phenol glycolipid-I [PGL-I]. The ML Flow is a one-step colloidal gold immune assay. \textit{M. leprae}-specific antigen is immobilised as a discrete line on a porous nitrocellulose membrane located in the test zone. The detection reagent consists of mobile red colloidal gold particles labelled with anti-human IgM which are deposited within the device. A sample of blood or serum is placed in the sample port and is carried with the running buffer. The detection reagent will attach to IgM antibodies in the sample and move through the porous membrane to the test zone. If an IgM antibody specific for the immobilised antigen is present they will bind and a red line will appear.

The testing method is as follows:

1. Spot 5 µl of serum onto the pad in the round sample port.
2. Add 130 µl running buffer to the round sample port.
A colour moving across test and control zones will be visible. Read results at 10 minutes. A negative result is indicated by absence of a line at the test zone and presence of a line at the control zone. A positive result is indicated by the presence of a strong line at the test zone and a line at the control zone. A strong positive staining intensity among close contacts of leprosy patients is suggestive of incubating multibacillary (MB) leprosy. A faint signal may indicate very low levels of specific antibody. In particular in endemic areas the presence of low specific antibody levels may correspond to antibodies present due to previous exposure and should be regarded as negative.

Results

The surveys were carried out between May and August 2005. The clinical and serological examinations were conducted in 398 people aged 3–84 years (average 50 years), comprising 196 males (49·2%) and 202 females (50·8%). The make-up of the study population was 293 ex-patients and 105 household members.

The results of the ML Flow in the eligible contacts and ex-patients showed that 18 people (4·5%) were seropositive, six people (1·5%) strongly positive and 374 people (94·0%) seronegative.

All six people who were strongly seropositive were ex-patients. Details of those six ex-patients are given in Table 1.

They were all then invited to the leprosy clinic for a physical examination for evidence of active leprosy. Five ex-patients (patient nos. 1–5) had no signs of active leprosy. Slit skin smears taken from these people were negative. Because of the possibility of MB relapse among ex-patients harbouring very high anti-PGL I Ab titers,\textsuperscript{5} MDT-MB has been reinstituted in these patients.

Patient no. 6, who was diagnosed with BL leprosy in 1986 with an initial bacterial index (BI) = 4·16 and then treated with MDT-MB for 7 years until his BI was negative had multiple red swollen papules, coalescing plaques on upper limbs, back and buttocks, present for a few months. Those skin eruptions did not respond to a topical corticosteroid cream. The skin lesions, hands and even feet became more swollen. His slit skin-smear examination had an average BI of 2·25, morphological index (MI) was nil. Relapsed BL with reversal reaction was diagnosed, MDT-MB and prednisolone 40 mg daily was started. The reactional state resolved within a week.

Table 1. Details of ex-patients who were strongly seropositive

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Type</th>
<th>Sex</th>
<th>Age</th>
<th>Previous Rx</th>
<th>Period of Rx</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>BL</td>
<td>F</td>
<td>53</td>
<td>MDT</td>
<td>1992–1995</td>
</tr>
<tr>
<td>2</td>
<td>TT</td>
<td>F</td>
<td>57</td>
<td>DDS</td>
<td>1973–1999</td>
</tr>
<tr>
<td>3</td>
<td>LL</td>
<td>M</td>
<td>73</td>
<td>DDS</td>
<td>1976–1987</td>
</tr>
<tr>
<td>4</td>
<td>LL</td>
<td>M</td>
<td>60</td>
<td>DDS</td>
<td>1962–1982</td>
</tr>
<tr>
<td>5</td>
<td>LL</td>
<td>M</td>
<td>49</td>
<td>DDS + B663</td>
<td>1979–1981</td>
</tr>
<tr>
<td>6</td>
<td>BL</td>
<td>M</td>
<td>73</td>
<td>MDT</td>
<td>1986–1993</td>
</tr>
</tbody>
</table>
The 18 people who were seropositive consisted of three normal household contacts, one ex-patient treated with MDT, and 14 ex-patients treated with DDS monotherapy.

Discussion

The introduction and full coverage of multidrug therapy (MDT) of registered cases of leprosy, has resulted in a sharp decrease in the number of registered and new leprosy patients in the world. At this level of coverage, the impact on leprosy transmission which is expected to be reflected in lower new case detection is likely to be limited in many areas as the declining prevalence of leprosy has not been matched by a declining new case detection rate. Leprosy control programs face a major problem of the existence of hidden unregistered and untreated cases. The assumption in leprosy control is that treatment of patients with MDT will reduce or eliminate the source of infection and thus prevent the transmission of the disease. However, widespread use of MDT has not yet prevented continued transmission of Mycobacterium leprae. Transmission that took place before the case finding exercise is therefore likely to result in many more new cases in future.

Apart from undetected or hidden leprosy patients, other major sources of ongoing transmission are likely to be those who are infected subclinically with M. leprae; especially people incubating multibacillary disease. There is increasing evidence from nasal PCR studies that subclinical transmission may exist and that those infected may go through a transient period, not resulting in disease development, but allowing transmission of infection to other individuals by nasal excretion.

Our study failed to confirm the hypothesis that there was an increased rate of subclinical M. leprae infection in Phra-Pradaeng Colony. In this report, 4.5% of the study population (15 ex-patients, and three contacts) were possibly subclinically infected patients, 1.5% (six ex-patients) possibly incubating multibacillary disease, thus demographic characteristics and previous treatment details were sought out in these six ex-patients. Of the five ex-patients who were possibly incubating multibacillary disease, one of them had completed MDT, while the other four had completed non-MDT regimens. MDT-MB has been re instituted in these patients to prevent development of clinical disease, although there is no controlled clinical trial data to support giving this treatment. It is interesting that based on the annual clinical examinations of ex-patients and contacts in the colony, as of September 2007, relapse or any new case has not yet been detected in the 398 study population.

Patient no. 6, the ex-patient who completed 7 years of MDT-MB until smear negativity had clinical relapse. It is not possible to differentiate between the possibilities of him relapsing from untreated M. leprae from his original infection which persisted despite 7 years of MDT, or whether he has been re-infected since he lives in a highly endemic area. Measurement of antibodies to PGL-1 can assist in identifying people with subclinical infection, and in this patient, clinical relapse could be confirmed.

In this study, 24 people were seropositive. In Indonesia, people who are seropositive have a 3-8 times higher risk of developing leprosy than negative people. In a recent published cohort study of household contacts of MB patients in the Philippines with a follow-up time of 11 years, seropositive contacts had a seven times higher risk of developing leprosy and a 24 times higher risk of developing MB leprosy. Household contacts, neighbours, and social contacts have a higher chance of contracting the disease. Whether this is mainly the result of closer contacts to the index case of the infection, similar genetic and immunological background, environmental
factors, or a combination of all, is not yet resolved. A large total-population study in Malawi identified household or dwelling contact as a risk factor: contacts had twice the risk compared with non-contacts. Contacts of MB patients had the highest risk: 5–8 times higher compared to non-contact. In the same cohort good housing conditions, education and a BCG scar was associated with a decreased risk of developing leprosy. Incident cases appear to emerge in clusters in the leprosy colony. Control of transmission may be feasible through identification of subclinical infection by appropriate diagnostic methods and treatment of individuals within infection clusters, improved housing conditions, sanitation and education, allowing progress towards eradication of leprosy.

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This research study was approved by the Ethical Committee of the Department of Disease Control, Ministry of Public Health, the Royal Thai Government, on May 27, 2004.

References