Mycobacterium leprae Particle Agglutination in Diagnosis and Monitoring of Treatment of Leprosy

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Abstract

IgM antibody levels against PGL-1 antigen were measured by *M.leprae* particle agglutination (MLPA) in 156 untreated leprosy patients. The seropositivity rate was much higher in newly untreated MB patients (84.7%) than in PB patients (19.7%). The mean MLPA titers in MB and PB declined significantly after 1 month of MDT ($p < 0.001$). Seropositivities in control serum specimens were 11.3 per cent in active pulmonary tuberculosis patients, 2.6 per cent in dermatologic patients and 4.4 per cent in a healthy population, in low titers.

The study confirms that, anti PGL-1 assay using MLPA is a sensitive and specific diagnostic tool for the diagnosis of leprosy especially MB patients. Additionally, it provides an alternative tool in monitoring leprosy patients under MDT.

Key word : Leprosy, MLPA, Diagnosis, Monitoring

The main strategy for controlling leprosy relies on early detection and effective treatment with multidrug therapy[1]. The development of a simple and rapid diagnostic test for leprosy is an important tool for the identification of those who are incubating the disease and for diagnosis in patients with atypical signs. Early treatment of this group would be able to prevent deformities and reduce the risk of spreading the disease to the community.

Several studies have been carried out to identify the specific antigenic determinants on the cellwall of *Mycobacterium leprae*[2]. One of these, phenolic glycolipid -1 (PGL-1) has been extensively studied[3,4]. The trisaccharide segment of the PGL-1 has been synthesized in the form of natural trisaccharide - phenyl propionate - bovine serum albumin (NT-P-BSA)[5,6]. This synthetic antigen has then been tested for its reactivity and specificity to leprosy sera by enzyme-linked immunosorbent assay (ELISA) and by *M.leprae* particle agglutination using gelatin particles (MLPA)[7-9], and the results are promising. Although ELISA is a specific and sensitive method, it is expensive and rather sophisticated. However, MLPA, using Serodia - Leprae (Fujirebio Inc., Tokyo, Japan) is more sim-

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ple, reliable and inexpensive that it can be carried out in the field and in countries where facilities are limited.

The objectives of this study were to conduct a seroepidemiological survey of anti PGL-1 antibody titers in new cases of leprosy and to study the change in antibody titers in leprosy patients during treatment.

MATERIAL AND METHOD

Study populations

Leprosy patients from Raj-Pracha-Samasai Institute, Bangkhaen Skin Clinic, Bangkok Skin Clinic and Phra-Pradaeng Hospital were included. Classification was done according to the Ridley-Jopling scale[10] as lepromatous (LL), borderline lepromatous (BL), mid borderline (BB), borderline tuberculoid (BT), tuberculoid (TT) and indeterminate (I). Multibacillary (MB) patients were defined as BT, BB, BL or LL patients having a bacteriological index (BI) of > 0; whereas BT, TT or I patients with a BI of 0 were defined as paucibacillary (PB). All recruited leprosy patients were assessed before treatment with the WHO recommended regimen of multidrug therapy[1]. In this regimen, PB cases received supervised rifampicin 600 mg once monthly and dapsone 100 mg everyday for at least 6 months. MB cases received supervised rifampicin 600 mg once monthly, supervised clofazimine 300 mg once monthly, clofazimine 50 mg once daily, and dapsone 100 mg once daily for at least 2 years.

Controls included active pulmonary tuberculosis patients from the Tuberculosis Division, Department of Communicable Disease Control, patients presented with skin diseases other than leprosy at Bangkhaen Skin Clinic and healthy individuals from the Department of Preventive Medicine, Faculty of Medicine, Siriraj Hospital, Mahidol University.

Detection of antibodies to PGL-1

A volume of 5 ml whole blood was drawn from each patient before starting chemotherapy and controls. The serum obtained was kept at -70°C until analysis with MLPA for the presence of anti-PGL-1 antibody as follows:

1. Qualitative assay. Serum dilutions of 1:8 and 1:16 were prepared by depositing 3 drops (75 ul) of serum diluent in well 1 and 1 drop (25 ul) each in well 2 and 3 of a 96-well U-type microtiter plate using a calibrated pipette dropper. 25 ul of each serum specimen was placed in well 1 and throughly mixed. 25 ul was transferred from well 1 to well 2, and subsequently from well 2 to well 3. The excess 25 ul from well 3 was discarded. One drop of unsensitized particles was added to well 2 and 1 drop of sensitized particles was added to well 3 using the droppers supplied in the kit. A microplate mixer was used to mix the fluid of the wells thoroughly. Plates were covered and allowed to stand at room temperature for 2 hours. Upon completion of the reaction the settling patterns were read with the naked eye. Specimens which were reactive with sensitized particles (showing a significantly large ring with a rough outer margin and agglutination in the periphery, or with a filmy mat of homogeneous agglutination covering the entire bottom of the well) and which were also nonreactive with unsensitized particles (showing compact button or compact ring with a smooth round outer margin) were interpreted as positives in the qualitative test.

2. Quantitative test. A semiquantitative test was performed by further serial two fold dilution to find the end point of the positive reaction. The antibody titer was expressed as the highest dilution giving complete agglutination. In this study, 1:32 was established as a cut-off value, defining a serum specimen which showed agglutination with less than 1:32 final serum dilution negative and that with equal to or more than 1:32 positive. If a pretreatment serum specimen was seropositive then antibody titers of subsequent specimens at 1, 3, 6, 12, 18 and 24 months after initiating treatment would be measured.

Data analysis

The seropositivity rates and geometric means ± standard deviation of antibody titers were calculated for each type of leprosy before chemotherapy. The change of mean antibody titers over time in MB and PB patients were analyzed by ANOVA if homogeneity of variance was fulfilled. If not, the Kruskal-Wallis ANOVA was used.

RESULTS

A total of 156 untreated leprosy patients aged 5 - 75 years (average 37.1 years) were entered into this study, with the male:female ratio being 2.0. The patients were classified into 51 LL, 26 BL, 1 BB, 7 BT-MB, 46 BT-PB, 18 TT and 7 I. The results of seropositivity rates among pretreatment speci-

Table 1. Seroreactivity to PGL-1 in leprosy patients before MDT.

Type	No. cases	Seropositive to PGL-1		
		No.	%	Range
MB(LL)	51	47	92.2	0-8192
MB(BL)	26	19	73.1	0-4096
MB(BB)	1	0	0	0
MB(BT)	7	6	85.7	0-512
Total	85	72	84.7	0-8192
PB(BT)	46	11	23.9	0-256
PB(TT)	18	1	5.6	0-32
PB(I)	7	2	28.6	0-32
Total	71	14	19.7	0-256

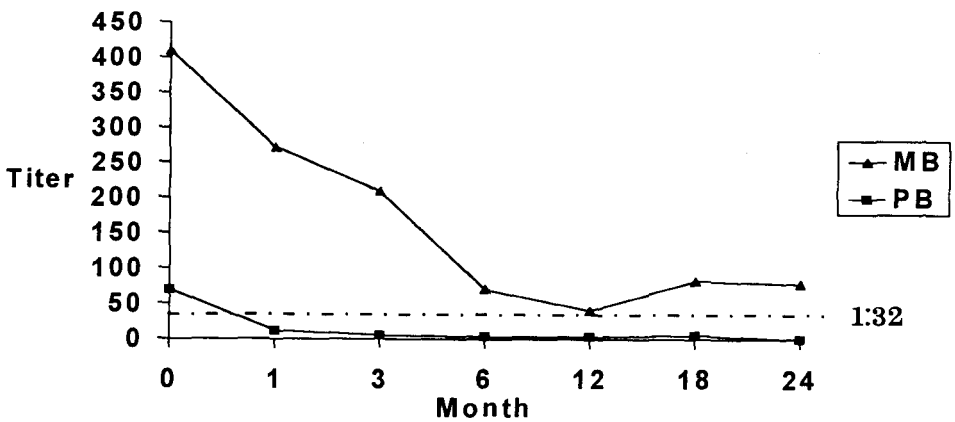

Fig. 1. The change of anti PGL-1 Ab in leprosy patients during treatment.

mens (Table 1) show that 72 out of 85 MB cases (84.7%) and 14 out of 71 PB (19.7%) were seropositive. High antibody titers up to 8,192 were found in 47 LL (92.2%). Titers up to 4,096 were found in 19 BL (73.1%), up to 512 in 6 BT-MB (85.7%), up to 256 in 11 BT-PB (23.9%), 32 in 2 I (28.6%) and 1 TT patient (5.6%). Only one BB case was seronegative.

The mean titer ± standard deviation before initiating treatment was 409.3 ± 4.4 in MB and 69.7 ± 2.1 in PB. The mean titer in MB decreased to 271.0 ± 8.4 at 1 month, 208.0 ± 8.2 at 3 months, 70.1 ± 10.6 at 6 months, 39.8 ± 11.7 at 12 months, 81.8 ± 4.1 at 18 months and 77.6 ± 5.4 at 24 months. In PB the mean titer rapidly converted seronegativity after 1 month of therapy (Fig. 1). Table 2 shows results in controls. Of 468 active pulmonary tuberculosis patients, 53 (11.3%) were positive by MLPA. Two dermatologic patients (2.6%) and 8 healthy persons (4.4%) were also seropositive in low titers (32-64).

DISCUSSION

Antibodies to PGL-1 antigen reflect a specific marker of *Mycobacterium leprae* infection (11-14). In this study, IgM antibody to semisynthetic trisaccharide moiety of PGL-1 was examined in

Table 2. Seroreactivity to PGL-1 in controls.

Type	No. cases	Seropositive to PGL-1	
		No.	%
TB	468	53	11.3
Dermatologic dis.	77	2	2.6
Healthy	179	8	4.4

newly diagnosed, untreated patients by MLPA. The percentages of seropositivity were much higher in MB (84.7%) than in PB patients (19.7%). The seropositivity rates for each type of leprosy were 92.2 per cent in LL, 73.1 per cent in BL, 85.7 per cent in BT-MB, 23.9 per cent in BT-PB, 28.6 per cent in I and 5.6 per cent in TT. Thus, a direct correlation has been found between the antibody titer and the severity of leprosy. Specificity of MLPA has also been supported by the impressive results in control samples; 11.3 per cent of TB patients, 2.6 per cent of dermatologic patients and 4.4 per cent of healthy individuals were tested positive with mostly low titers (32-64). This demonstrates that IgM antibody to PGL-1 detected by MLPA is a useful marker for seroepidemiological surveys and can assist in the diagnosis and possible classification of leprosy, particularly MB cases. MLPA was also evaluated as a useful tool for monitoring leprosy patients under treatment. The mean antibody titer in MB and PB cases declined significantly after 1 month of therapy ($p<0.001$). It is a good complement to clinical observation and serial BI determination, which are subjective and insensitive. The mouse footpad technique is the only available tool for testing response to treatment, nevertheless it is too invasive, labor-intensive and time-consuming.

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การตรวจ MLPA เพื่อการวินิจฉัยและติดตามการรักษาโรคเรื้อน

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คณะผู้วิจัยได้ใช้ MLPA ในการวัดระดับแอนติบอดี้ต่อ PGL-1 ในผู้ป่วยโรคเรื้อนในเขตกรุงเทพมหานครและปริมณฑล จำนวน 156 ราย พบว่าในผู้ป่วยใหม่ที่ไม่เคยรักษาชนิดเชื้อมาก (MB) มีผลบวก 84.7% และ 19.7% ในผู้ป่วยชนิดเชื้อน้อย (PB) จากการติดตามวัดระดับแอนติบอดี้เปรียบเทียบก่อนและระหว่างรักษา พบว่าค่าเฉลี่ยลดลงอย่างมีนัยสำคัญทางสถิติ ($p<0.001$) ที่ 1 เดือนหลังเริ่มรักษา การศึกษาในกลุ่มควบคุมพบแอนติบอดี้ระดับต่ำ ๆ ใน 2.6-11.3% ของประชาชนทั่วไป, ผู้ป่วยโรคผิวหนังและผู้ป่วยวัณโรคปอดระยะติดต่อ สรุปว่าในผู้ป่วยโรคเรื้อนที่ไม่เคยรักษาการวัดระดับแอนติบอดี้ต่อ PGL-1 มี sensitivity และ specificity สูงซึ่งมีประโยชน์ช่วยในการวินิจฉัยและติดตามผลการรักษาโดยเฉพาะผู้ป่วยชนิด MB

คำสำคัญ : โรคเรื้อน, เอ็มแอลพีเอ, การวินิจฉัย, การติดตาม

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