Early detection of leprosy by examination of household contacts, determination of serum anti-PGL-1 antibodies and consanguinity

Renata Bazan-Furini¹, Ana Carolina F Motta¹, João Carlos L Simão¹, Daniela Chaves Tarquínio¹, Wilson Marques Jr², Marcello Henrique N Barbosa³, Norma Tiraboschi Foss¹

¹Divisão de Dermatologia ²Divisão de Neurologia ³Divisão de Radiologia, Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, Av. Bandeirantes 3900, 14049-900 Ribeirão Preto, SP, Brasil

A cross-sectional clinical trial in which the serum anti-phenolic glycolipid (anti-PGL-1) antibodies were analysed in household contacts (HHC) of patients with leprosy as an adjunct early leprosy diagnostic marker was conducted. The families of 83 patients underwent clinical examination and serum anti-PGL1 measurement using enzyme-linked immunosorbent assay. Of 320 HHC, 98 were contacts of lepromatous leprosy (LL), 80 were contacts of borderline lepromatous (BL), 28 were contacts of borderline (BB) leprosy, 34 were contacts of borderline tuberculoid (BT), 40 were contacts of tuberculoid (TT) and 20 were contacts of indeterminate (I) leprosy. Consanguinity with the patients was determined for 232 (72.5%) HHC. Of those 232 contacts, 183 had linear consanguinity. Forty-nine HHC had collateral consanguinity. Fifty-eight contacts (18.1%) tested positive for anti-PGL1 antibodies. The number of seropositive contacts based on the clinical forms of the index case was 17 (29.3%) for LL, 15 (25.9%) for BL, one (1.7%) for BB, 14 (24.1%) for BT, three (5.2%) for TT and eight (13.7%) for I. At the one year follow-up, two (3.4%) of these seropositive contacts had developed BT leprosy. The results of the present study indicate that the serum anti-PGL-1 IgM antibody may be useful for evaluating antigen exposure and as a tool for an early leprosy diagnosis in HHC.

Key words: leprosy - household contacts - early detection - anti-PGL-1 - consanguinity

Leprosy is a chronic infectious disease caused by Mycobacterium leprae, an acid-fast bacillus that presents a peculiar tropism for peripheral nerves and the skin. The prevalence of leprosy in the world has declined since the introduction of the multidrug therapy recommended by the World Health Organization (WHO 1982). However, leprosy is still a public health problem, especially in Brazil, where the number of new cases is high (37,610 cases in 2009) (WHO 2010).

Significant progress has been made in controlling leprosy and reducing the burden of the disease; however, there is much that is still required to reduce the disease burden. Early case identification is one of the aims of leprosy control programs (Oskam et al. 2003, WHO 2009). Thus, controlled clinical trials conducted on household contacts (HHC) are of great importance for the detection of new disease cases (Foss et al. 1993, Goulart et al. 2008). With this idea in mind, we undertook the study of examining the anti-PGL-1 antibody levels in HHC of leprosy patients.

Leprosy diagnosis is based on clinical examination, bacterial index, histopathological findings (Ridley & Jopling 1966) and the serologic determination of antibody bodies. The phenolic glycolipid antibody (PGL-1), as measured using an enzyme-linked immunosorbent assay (ELISA), is considered to be a relevant marker of leprosy activity (Burgess et al. 1988, Zenha et al. 2009, Frota et al. 2010). The PGL-1 fraction is part of the cell envelope of M. leprae and induces the production of the specific humoral response against PGL-1 detected in patient serum (Hunter et al. 1982, Cho et al. 1983, Foss et al. 1993). When the antibody is present at high levels, it can be inferred that the infection is active, especially during the reactional episodes that constitute a very common complication in the evolution of leprosy (Chin-A-Lein et al. 1992, Goulart et al. 2002).

With the current emphasis on strategies leading to an early diagnosis of leprosy in the world (WHO 2009), we need laboratory markers to detect leprosy in patients during the early stages of the disease and to help reduce the disability and deformities caused by permanent nerve damage. Thus, the present cross-sectional clinical trial was conducted to analyse the serum titres of anti-PGL-1 antibodies as an adjunct tool for early leprosy diagnosis among leprosy HHC.

PATIENTS, MATERIALS AND METHODS

Patients - The families of leprosy patients being treated at the Leprosy Clinics of the School of Medicine of Ribeirão Preto (HCRP), University of São Paulo, were recruited. Those who agreed to participate in the study were rescheduled for a clinical examination (nerve thickness, cutaneous lesions and sensitivity) by an experienced examiner and for serum anti-PGL-1 measurement. Subjects were excluded if they presented with co-existing local or...
systemic infection and/or any disease that could affect
the peripheral nervous system such as diabetes mellitus
and alcoholism. The trial was approved by the local Ethni-
cal Committee (HCRP 13.549/2005) and all subjects pro-
vided written informed consent to participate.

Serum antibody titres - Ninety-six-well polystyrene
plates (Costar, Cambridge, USA) were coated with anti-
ogen (PGL-1, kindly provided by Dr JS Spencer, Colorado
University, USA) in sodium carbonate buffer (2 µg/mL),
PH 9.6 and stored at 4°C until used. The serum from
each patient was diluted 1:100 in 15 mM Tris-Tween
buffer containing 5% sheep serum. 10 µL were added to
each well and the plate was incubated for 1 h at 37°C in
a humidified chamber. At the end of the hour, the samples
were washed with 15 mM Tris-Tween buffer and anti-
human IgM beta-galactosidase conjugate (Sigma, USA)
diluted 1:600 in 15 mM Tris-Tween buffer containing
5% sheep serum. The plates were incubated at 37°C for
1 h. A fluorogenic substrate (10 µL 4-methylumbelliferyl
beta-D-galactopyranoside) was then added to the sam-
ple and the material was incubated at 37°C for 30 min.
The plate was read with a multiscan ELISA reader. Sera
samples with an absorbance at 450 nm greater than 0.028
[the mean absorbance plus 3 standard deviations (SD) of
40 healthy Brazilian control subjects] were considered
positive. Each serum sample was tested in duplicate.

Follow-up - All HHC have received follow-up ex-
aminations at the Leprosy Clinics at six-month inter-
vals since 2005.

Statistical analysis - Odds ratios and a Fisher’s exact
test were used to test for differences between the groups
that presented anti-PGL-1 levels ≥ or < cut-off (positive
and negative levels, respectively) with the aid of the Sta-
tistical Analysis System - SAS® 9.0 software (San Diego,
Cary, NC, USA). Significance was set at p < 0.05.

RESULTS

Characteristics of the study population - Eighty-three
families agreed to participate in the study. Of these fami-
ilies, 331 HHC were examined and 11 were diagnosed as
leprosy patients: two patients presented lepromatous lep-
rosy (LL), four presented the tuberculoid form (TT) and
five presented the indeterminate form (I). The final study
population sample enrolled consisted of 320 HHC of lep-
rosy patients (130 men and 190 women, mean ± SD age
27.01 ± 19.57 years, range 1-81 years). HHC were grouped
according to anti-PGL-1 levels. The characteristics of the
patients are listed in Table I. Among the 83 leprosy pa-
tients, 25 presented LL, 20 presented borderline lepro-
matous (BL), seven were borderline (BB), 16 were borderline
tuberculoid (BT), nine were TT and six were I.

Profile of HHC - Ninety-eight HHC were contacts of
LL patients, 80 of BL patients, 28 of BB patients, 54 of
BT patients, 40 of TT patients and 20 of I leprosy pa-
tients. Two-hundred-and-thirty-two HHC had some level
of relationship, i.e., linear consanguinity with the index
case in 183 contacts and collateral consanguinity in 49
contacts. The other 88 HHC had an affinity relationship.

Serum antibody titres - Of the 320 contacts, 58 (18.1%) had
positive anti-PGL-1 levels. The anti-PGL-1 sero-
positivity levels among the contacts based on the clinical
forms of the index case inside the household were 17
(29.3%) for LL, 15 (25.9%) for BL, one (1.7%) for BB, 14
(24.1%) for BT, three (5.2%) for TT and eight (13.7%) for
I. Serum anti-PGL-1 IgM antibodies were higher among
female HHC (35) than among male HHC (23) (Table II).

Follow-up - All of the HHC are being followed at
six-month intervals at the Leprosy Clinics, especially
the seropositive contacts. After one year of follow-up,
two seropositive contacts with confirmed linear consan-
guinity developed BT leprosy.

DISCUSSION

In the present study, we first evaluated HHC of lep-
rosy patients and detected 11 new leprosy cases (3.3% of
the contacts) based on the clinical examination. The
cases were later confirmed with histopathological exam-
ination and bacilloscopic index, emphasising the impor-
tance of examining HHC for an early diagnosis (5 cases
of I) and for the detection of occult prevalence (2 cases of
LL) in a non-endemic region, as the leprosy prevalence
in Ribeirão Preto is 0.89/10,000 habitants in 2009 (SI-
NAN 2010). Similar results were reported by Cardona-
Castro et al. (2005), who detected two multibacillary
(MB) leprosy cases among the HHC from a Colombian
region considered to be in the post-elimination phase.
Therefore, we emphasise that the evaluation of HHC is a
useful tool for leprosy control not only in endemic areas,
but also in non-endemic areas.

In the present study, in an attempt to improve early
detection, the levels of anti-PGL-1 antibodies were as-
essed in serum samples from HHC of leprosy patients,
as an adjunct to the determination of antigen exposure.
The median age was similar for both seropositive and
seronegative cases. The proportion of anti-PGL-1-sero-
positive HHC among the different clinical forms was dif-
f erent from the proportion previously reported in some
studies conducted in endemic areas (Gonzalez-Abreu et
these studies found no differences in seropositivity rates
among HHC of MB and paucibacillary leprosy (PB) lep-
rosy patients, our results indicated a slight difference
in serum anti-PGL-1 IgM seropositivity rates between
HHC of MB leprosy patients (56.8%) and of PB leprosy
patients (43.1%), especially when comparing I leprosy
patients (14%) x LL leprosy patients (29%) (p = 0.03).
Similar results were reported by Calado et al. (2005),
Carbona-Castro et al. (2008) and Frota et al. (2010). The
presence of serum antibodies suggests that the PGL-1
antigen induced a humoral immune response, but these
increased antibody concentrations apparently could not
block M. leprae multiplication in the host (Kaplan
& Chase 1980, Touw et al. 1982), as we found that two
cases progressed to BT leprosy after one year of follow-
up of these seropositive contacts.

The results suggest a useful role for the measure-
ment of serum M. leprae-specific anti-PGL-1 IgM
antibodies. The measurement can be used as an easy,
### TABLE I
Clinical data of the leprosy patients and their household contacts (HHC)

<table>
<thead>
<tr>
<th>Variables</th>
<th>Index case n = 83 (%)</th>
<th>HHC n = 320 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>58 (69.8)</td>
<td>130 (40.6)</td>
</tr>
<tr>
<td>Female</td>
<td>25 (30.1)</td>
<td>190 (59.4)</td>
</tr>
<tr>
<td>Age Mean (range)</td>
<td>51.46 ± 14.60 (9-75)</td>
<td>27.01 ± 19.57 (1-81)</td>
</tr>
<tr>
<td>Clinical classification</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Indeterminate leprosy</td>
<td>6 (7.2)</td>
<td>20 (6.2)</td>
</tr>
<tr>
<td>Tuberculoid leprosy</td>
<td>9 (10.8)</td>
<td>40 (12.5)</td>
</tr>
<tr>
<td>Borderline tuberculoid</td>
<td>16 (19.2)</td>
<td>54 (16.8)</td>
</tr>
<tr>
<td>Borderline leprosy</td>
<td>7 (8.4)</td>
<td>28 (8.7)</td>
</tr>
<tr>
<td>Borderline lepromatous</td>
<td>20 (24)</td>
<td>80 (25)</td>
</tr>
<tr>
<td>Lepromatous leprosy</td>
<td>25 (30.1)</td>
<td>98 (30.6)</td>
</tr>
<tr>
<td>Operational classification</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Paucibacillary leprosy</td>
<td>31 (37.3)</td>
<td>114 (35.6)</td>
</tr>
<tr>
<td>Multibacillary leprosy</td>
<td>52 (62.6)</td>
<td>206 (64.4)</td>
</tr>
</tbody>
</table>

### TABLE II
Clinical data and anti-glycolipid antibody (PGL-1) titres of the household contacts (HHC) of leprosy patients

<table>
<thead>
<tr>
<th>Variables</th>
<th>HHC n (%)</th>
<th>Total</th>
<th>Odds-ratio (95% CI)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Anti-PGL-1 &lt; 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Anti-PGL-1 ≥ 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>107 (40.8)</td>
<td>23 (39.7)</td>
<td>130</td>
<td>1.155 (0.644-2.073)</td>
</tr>
<tr>
<td>Female</td>
<td>155 (57.2)</td>
<td>35 (60.3)</td>
<td>190</td>
<td>-</td>
</tr>
<tr>
<td>Age Mean (range)</td>
<td>26.64 ± 19.23 (1-81)</td>
<td>28.06 ± 19.72 (1-81)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Clinical classification of the index case</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Indeterminate leprosy</td>
<td>12 (4.5)</td>
<td>08 (13.7)</td>
<td>20</td>
<td>3.176 (1.127-8.953)</td>
</tr>
<tr>
<td>Tuberculoid leprosy</td>
<td>37 (14.1)</td>
<td>03 (5.2)</td>
<td>40</td>
<td>0.386 (0.107-1.400)</td>
</tr>
<tr>
<td>Borderline tuberculoid</td>
<td>40 (15.2)</td>
<td>14 (24.1)</td>
<td>54</td>
<td>1.549 (0.685-3.500)</td>
</tr>
<tr>
<td>Borderline leprosy</td>
<td>27 (10.3)</td>
<td>01 (1.7)</td>
<td>28</td>
<td>0.367 (0.079-1.694)</td>
</tr>
<tr>
<td>Borderline lepromatous</td>
<td>65 (24.8)</td>
<td>15 (25.9)</td>
<td>80</td>
<td>1.083 (0.503-2.331)</td>
</tr>
<tr>
<td>Lepromatous leprosy&lt;sup&gt;d&lt;/sup&gt;</td>
<td>81 (30.9)</td>
<td>17 (29.3)</td>
<td>98</td>
<td>-</td>
</tr>
<tr>
<td>Operational classification</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Paucibacillary leprosy</td>
<td>89 (34)</td>
<td>25 (43.1)</td>
<td>114</td>
<td>0.733 (0.410-1.312)</td>
</tr>
<tr>
<td>Multibacillary leprosy</td>
<td>173 (66)</td>
<td>33 (56.9)</td>
<td>206</td>
<td>-</td>
</tr>
<tr>
<td>Relationship with the index case</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Consanguinity&lt;sup&gt;c&lt;/sup&gt;</td>
<td>187 (70.6)</td>
<td>45 (81.8)</td>
<td>232</td>
<td>-</td>
</tr>
<tr>
<td>Linear&lt;sup&gt;d&lt;/sup&gt;</td>
<td>153</td>
<td>30</td>
<td>183</td>
<td>-</td>
</tr>
<tr>
<td>Collateral</td>
<td>34</td>
<td>15</td>
<td>49</td>
<td>2.129 (1.039-4.363)</td>
</tr>
<tr>
<td>Affinity&lt;sup&gt;e&lt;/sup&gt;</td>
<td>78 (29.4)</td>
<td>10 (18.2)</td>
<td>88</td>
<td>0.817 (0.397-1.681)</td>
</tr>
</tbody>
</table>

<sup>a</sup>: cut-off; <sup>b</sup>: clinical form; <sup>c</sup>: relationship by blood; <sup>d</sup>: relationship used as reference for comparison between the others; <sup>e</sup>: relationship by marriage; CI: confidence interval.
non-invasive and inexpensive adjunct method for the detection of leprosy in the population, as reported previously (Foss et al. 1993, Frota et al. 2010). In addition, the results suggest an important role for the measurement of serum anti-PGL-1 antibodies as a method for detecting subclinical infection, especially in leprosy contacts with low or no resistance to M. leprae (Foss et al. 1993). As seropositivity for IgM anti-PGL-1 might be a risk factor for developing leprosy, the seropositive individuals should be monitored via clinical examination, determination of the immune response and bacteriologic state for leprosy detection (Douglas et al. 2004, Cardona-Castro et al. 2009), as was done with the HHC selected in the present study.

There are some risk factors for the development of leprosy, in addition to being a contact, such as clinical form of leprosy of the index case, the physical distance and the consanguinity (Richardus et al. 2005, Moet et al. 2006). Consanguinity is the relationship between members from the same family and it can be classified as linear and collateral. Linear consanguinity is the blood relationship that exists among persons, where one person is descended from the other and proceeds upwards in a direct ascending line, whereas collateral consanguinity is the relation subsisting among persons who are descended from the same common ancestor, but not from each other. Affinity was considered to be a relationship by marriage. The importance of consanguinity for the development of anti-PGL-1 IgM antibodies should be emphasised as most of the contacts with positive anti-PGL-1 titres (45/58) had a family history of leprosy. In addition, the two cases that developed BT leprosy had a confirmed linear consanguinity with the index case.

The results of this study indicate that in a low-prevalence area a high proportion of HHC of leprosy patients are at an increased risk of developing leprosy (Cardona-Castro et al. 2005, 2008, Dessunti et al. 2008). In conclusion, the clinical evaluation and serum measurements of anti-PGL-1 IgM are useful for the evaluation of antigen exposure. The follow-up of HHC with high levels of anti-PGL-1 IgM could facilitate the characterisation of those contacts who are at risk of developing leprosy, particularly in cases of linear consanguinity.

ACKNOWLEDGMENTS

To the authors, for the participation of the patients and communities, and to Mr Mario Ignácio Neto, for assistance with sample analysis.

REFERENCES


Early detection of leprosy • Renata Bazan-Furini et al. 539
are independent risk factors for leprosy in contacts of patients with leprosy. *J Infect Dis* 193: 346-353.


