Clinical and Immunological Aspects of Post–Kala-Azar Dermal Leishmaniasis in Bangladesh


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Abstract. We conducted active surveillance for kala-azar and post–kala-azar dermal leishmaniasis (PKDL) in a population of 24,814 individuals. Between 2002 and 2010, 1,002 kala-azar and 185 PKDL cases occurred. Median PKDL patient age was 12 years; 9% had no antecedent kala-azar. Cases per 10,000 person-years peaked at 90 for kala-azar (2005) and 28 for PKDL (2007). Cumulative PKDL incidence among kala-azar patients was 17% by 5 years. Kala-azar patients younger than 15 years were more likely than older patients to develop PKDL; no other risk factors were identified. The most common lesions were hypopigmented macules. Of 98 untreated PKDL patients, 48 (49%) patients had resolution, with median time of 19 months. Kala-azar patients showed elevated interferon-γ (IFN-γ), tumor necrosis factor-α (TNF-α), and interleukin 10 (IL-10). Matrix metalloproteinase 9 (MMP9) and MMP9/tissue inhibitor of matrix metalloproteinase-1 (TIMP1) ratio were significantly higher in PKDL patients than in other groups. PKDL is frequent in Bangladesh and poses a challenge to the current visceral leishmaniasis elimination initiative in the Indian subcontinent.

INTRODUCTION

Post–kala-azar dermal leishmaniasis (PKDL) is a dermatosis seen after apparently successful treatment of visceral leishmaniasis (VL; kala-azar).1 PKDL is more common in areas with *Leishmania donovani* than in areas where VL is caused by *L. infantum*. In Sudan, PKDL occurs in up to 60% of VL patients, usually within 6 months after VL onset; a large percentage is mild and resolves without antileishmanial treatment.1–3 In South Asia, PKDL is reported to occur in 5–10% of VL patients, with an interval of 2–3 years between VL and PKDL, and published dogma holds that treatment is required for PKDL resolution.1,4 Although molecular techniques have improved diagnostic sensitivity, these assays are generally limited to research settings, and most cases are still diagnosed based on clinical features.1,5

In 2007, we initiated a study in a group of villages in Bangladesh.6 The design included identification of kala-azar and PKDL cases through an initial retrospective survey followed by prospective surveillance to assess incidence and duration of the two conditions and identify risk factors for development of PKDL after kala-azar. A preliminary analysis showed a rising incidence of PKDL from 2002 to 2008.6 Systematic examination of PKDL patients was performed to describe the spectrum of the disease in the community. Finally, serum specimens were collected from a subset of kala-azar and PKDL patients with the aim of describing cytokine profiles.

MATERIALS AND METHODS

Survey. The study was conducted in Fulbaria, Mymensingh, in villages selected for high reported case numbers in government data. The protocol was approved by the ethics committees of icddr,b and Centers for Disease Control and Prevention (CDC). The baseline house-to-house survey was conducted from July of 2007 to March of 2008. After informed consent, field teams collected census data and ascertained current and past kala-azar and PKDL. Field workers were trained by the study physician to recognize characteristic PKDL lesions. Potential PKDL cases were examined by the physician, and findings were recorded on a structured form. Written informed consent, including consent for publication in a journal, was obtained before taking photographs.

After the baseline survey, a health education campaign was conducted to raise awareness of kala-azar and PKDL. Subsequently, fieldworkers visited each community on a consistent day of the month to search for new cases. Participants were urged to self-report rashes or febrile illness lasting 2 weeks or more. House-to-house searches were conducted on three subsequent occasions (January to March of 2009, January to March of 2010, and October to December of 2010) to seek new cases and ascertain status of known kala-azar and PKDL cases using questionnaires and when indicated, examination by the physician. Resolution of PKDL lesions was confirmed by physician examination. Surveillance concluded in December of 2010.

Case definitions. For most patients, PKDL was diagnosed based on examination for characteristic clinical features by an experienced physician. The definition required stable presence of lesions consistent with PKDL for at least 1 month and exclusion of other conditions, including leprosy, fungal dermatoses, and vitiligo.4,7 Cases with mild or ambiguous findings were followed for at least 2 months; cases in which lesions remained ambiguous or an alternative diagnosis was made were excluded. Lesions were classified as macules (flat, non-palpable, and hypopigmented), papules (raised and less than 0.5 cm in diameter), plaques (raised and more than 0.5 cm in diameter), and/or nodules (palpable, firm, and rounded).8 PKDL extent was categorized by presence or absence in four sites: face, torso, arms, and legs. Patients with active disease were given referral letters to the local government hospital.

Laboratory methods. During the baseline survey, blood specimens (3-mL tube with ethylene-diamine-tetra-acid and 3-mL tube without additive) were collected from participants newly diagnosed with PKDL or kala-azar. A small number of PKDL patients also had skin specimens collected and placed in phosphate-buffered saline solution. In 2010, a subgroup had follow-up blood specimens, including 11 specimens
from resolved PKDL patients and 15 specimens from cured kala-azar patients. Specimens were centrifuged, separated, transported on ice to icddr,b, and stored at −20°C.

DNA was extracted from blood using the QIAamp DNA MiniKit (Qiagen, Hilden, Germany) following the manufacturer’s instructions. Slit skin specimens underwent proteinase K digestion at 50°C for 60 minutes followed by the same extraction method. DNA was amplified using a nested polymerase chain reaction (PCR) targeting the small subunit rRNA of *L. donovani* complex.9

Levels of serum cytokines (interleukin 2 [IL-2], −4, −5, −10, −12, −13, interferon-γ [IFNγ], tumor necrosis factor-α [TNFα], and macrophage inhibitory protein 1α [MIP1α]), matrix metalloproteinase 1 [MMP1], −2, −7, −9, and −10, and tissue inhibitors of matrix metalloproteinase 1 [TIMP1] and −2 were assessed using multiplex assays and bead array technology. Serum was incubated with bead populations labeled with specified intensities of fluorescent dyes to create a unique spectral address and coated with capture antibodies as determined by each array (Millipore, Billerica, MA). Samples were incubated with antibody-coated beads overnight (cytokines) or for 2 hours (MMPs and TIMPs). Beads were washed and incubated with biotinylated detection antibody followed by a short incubation with streptavidin conjugated to a fluorochrome. Fluorescence was measured on a BioPlex suspension array system (BioRad, Hercules, CA), and analyte concentrations were calculated from a standard curve run concurrently on each plate. Sera from seven adult North American subjects were included as non-endemic controls.

**Statistical analysis.** Data were double-entered, cross-checked for errors, and cleaned by running logical cross-tabulations and checking against questionnaires. Descriptive analysis used χ², t, or Wilcoxon rank sum test as appropriate. Epidemic curves were calculated using a Nelson–Aalen estimate based on onset dates and person-time at risk.10 The monthly incidence was that month’s increment in the Nelson–Aalen estimate, which is equivalent to the number of new kala-azar or PKDL cases divided by the number of people at risk during that month accounting for migration, births, and deaths. We then fit a cubic smoothing spline11 to the Nelson–Aalen estimate and took the first derivative. Confidence intervals were based on Greenwood’s formula and a log transformation. Survival curves were constructed for PKDL incidence after kala-azar and PKDL resolution with and without treatment. To compare groups, we calculated Kaplan–Meier curves separately and tested the null hypothesis using a log-rank test.12,13 Differences in distribution of cytokines, MMPs, and TIMPs and the ratio of MMP9 to TIMP1 were evaluated by the Kruskal–Wallis test for each pair of groups. Analyses were performed in R (http://www.r-project.org/) and SAS 9.0 (SAS Institute Inc., Cary, NC).

**RESULTS**

The population included 24,814 individuals in 5,277 households in eight villages. A total of 1,002 participants reported kala-azar with onset between 2002 and 2010; an additional 293 kala-azar cases with onset between 1992 and 2001 were reported. Of 1,002 kala-azar patients, the first treatment course consisted of sodium stibogluconate (SSG) in 948 patients, miltefosine in 53 patients, and liposomal amphotericin B in 1 patient. A second treatment course was needed for 42 kala-azar patients: 26 (2.7%) of those patients treated with SSG compared with 16 (30.2%) of those patients treated with miltefosine (*P* < 0.0001). Among patients with documentation of miltefosine source, the failure rate was 1 in 28 (3.6%) participants treated in a clinical trial of brand name drug14 compared with 15 of 18 (83.3%) treated in 2008 with locally produced miltefosine (*P* < 0.0001 by Fisher exact test).

A total of 185 PKDL cases occurred with onset between 2002 and 2010; 172 active PKDL cases were identified during surveillance, and 13 cases of treated or resolved PKDL were reported retrospectively at the time of initial data collection. Overall, 165 (89%) participants reported having been treated for kala-azar before onset of PKDL. Three patients reported an interval of less than 3 months between kala-azar and PKDL, and they were treated for both conditions simultaneously; one patient had simultaneous onset (para–kala-azar dermal leishmaniasis). Seventeen (9.2%) PKDL patients reported no history of prior kala-azar. Median age at PKDL onset was 12 years (range = 2–68 years); 55% of patients were male.

The epidemic curve shows rising kala-azar incidence starting in 2002, with a peak of 90/10,000 person-years in 2005 followed a marked decline (Figure 1). PKDL incidence peaked in late 2007 at 28/10,000 person-years. The PKDL curve echoed the curve of kala-azar, with an offset of approximately 2 years. The median interval from kala-azar treatment completion to PKDL onset was 19 months (range = 0–120 months). The cumulative incidence of PKDL among kala-azar patients was approximately 3% within 1 year, 10% within 2 years, and 17% within 5 years (Figure 2A). Patients younger than 15 years at the time of kala-azar were significantly more likely than older patients to develop PKDL (log-rank *P* = 0.023) (Figure 2B). There was no difference in PKDL risk by sex (*P* = 0.54) or kala-azar treatment drug (*P* = 0.86), but data for drugs other than SSG were sparse.

Physical examination data were available for 138 PKDL patients (Table 1). Among patients with lesions when fieldwork
commenced, there was no difference by sex or age between those patients with and without examination data, but data were more likely to be missing for patients with onset before 2007 compared with patients with onset in 2007 or later ($P < 0.01$). Patients reported median PKDL duration of 7 months (range = 0–60 months) at the time of examination. Lesions were widespread, with 71% reporting lesions on at least two anatomical areas. The face, especially the perioral area (Figure 3A and B), tended to be the earliest site affected, with later spread to torso and limbs.

The most common lesions were hypopigmented macules; this lesion was the sole type in 45%, and it occurred with papules in an additional 23% of patients. Macules were sometimes large and coalescent. Pure papular disease affected the face alone in 30%, but more often, it affected the torso and/or limbs (Figure 3C and Table 2); 32 patients had complex lesions, 27 (19.6%) patients had plaques plus macules and/or papules, and 5 (3.6%) patients had nodules plus other lesion types (Figure 3D and E). Patients with complex lesions were older than other patients (median = 21 versus 11 years, $P = 0.02$), had a longer interval from kala-azar treatment to onset (27 versus 19 months, $P = 0.07$), and had a longer duration at the time of examination (12 versus 6 months, $P = 0.20$). PKDL duration at the time of examination increased significantly by increasing severity ($P = 0.04$ for severity score 2 versus 1; $P = 0.02$ for severity score 3 versus 1). PCR yielded positive results in $8 (34.8\%)$ of 23 patients tested; there was no difference by lesion type or severity, but the stratified numbers were small.

**Table 1**

<table>
<thead>
<tr>
<th>Clinical characteristics</th>
<th>N (%)</th>
<th>Mean severity score*</th>
<th>Proportion of males (%)</th>
<th>Median age (years)</th>
<th>Median duration of PKDL (months)$†$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Among all patients</td>
<td>138 (100)</td>
<td>2.1</td>
<td>57</td>
<td>12</td>
<td>7</td>
</tr>
<tr>
<td>Anatomical sites involved‡</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Face only</td>
<td>22 (16)</td>
<td>1.9</td>
<td>41</td>
<td>13</td>
<td>9</td>
</tr>
<tr>
<td>Face plus torso and/or limbs</td>
<td>53 (38)</td>
<td>1.9</td>
<td>55</td>
<td>12</td>
<td>5</td>
</tr>
<tr>
<td>Torso and/or limbs only</td>
<td>17 (12)</td>
<td>2.1</td>
<td>76</td>
<td>18</td>
<td>12</td>
</tr>
<tr>
<td>Face, torso, arms, and legs</td>
<td>46 (33)</td>
<td>2.6</td>
<td>61</td>
<td>11.5</td>
<td>11.5</td>
</tr>
<tr>
<td>Lesion types</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Macules only</td>
<td>62 (45)</td>
<td>2.2</td>
<td>60</td>
<td>10.5</td>
<td>6</td>
</tr>
<tr>
<td>Papules only</td>
<td>13 (9)</td>
<td>1.8</td>
<td>38</td>
<td>12</td>
<td>7</td>
</tr>
<tr>
<td>Macules and papules</td>
<td>31 (22)</td>
<td>2.2</td>
<td>61</td>
<td>13</td>
<td>6</td>
</tr>
<tr>
<td>Complex§</td>
<td>32 (23)</td>
<td>2.2</td>
<td>56</td>
<td>21</td>
<td>12</td>
</tr>
<tr>
<td>Severity score¶</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mild (1)</td>
<td>27 (20)</td>
<td>–</td>
<td>63</td>
<td>13</td>
<td>4</td>
</tr>
<tr>
<td>Moderate (2)</td>
<td>65 (47)</td>
<td>–</td>
<td>52</td>
<td>12</td>
<td>7</td>
</tr>
<tr>
<td>Severe (3)</td>
<td>46 (33)</td>
<td>–</td>
<td>61</td>
<td>11.5</td>
<td>10.5</td>
</tr>
</tbody>
</table>

*Mean severity scored by examiner as mild (1), moderate (2), or severe (3; based on the highest severity score reported for that patient).
†Months since onset of lesions at the time of examination.
‡Four sites evaluated: face, torso, arms, and legs.
§Complex lesions defined as plaques plus macules and/or papules or nodules plus any of the other three lesion types. Of 32 patients with lesions in this category, 27 patients had plaques plus macules and/or papules, and 5 patients had nodules plus macules, papules, and/or plaques. There were no patients with plaques or nodules who did not also have other lesion types.
¶The highest severity score among scores for each type of lesion in each anatomical site for the individual.
Eighty-seven PKDL patients were treated by the end of the study: 57 patients with SSG and 30 patients with liposomal amphotericin B. Patients 15 years or older were more likely to be treated than patients younger than 15 years (43/78 [55%] versus 44/107 [41%]; \( P = 0.06 \)); there was no difference by sex. SSG was the only drug used between September of 2003 and March of 2010. Patients reported receiving 56 to 128 injections; 42 (74%) patients reported having received 120 injections, consistent with national guidelines at the time. After April of 2010, all patients were treated by Médecins sans Frontières-Holland with liposomal amphotericin B at 5 mg/kg two times per week for 3 weeks. Among patients treated with SSG, median follow-up time after treatment was 29 months (7–73 months); 50% resolved by the end of the 6-month treatment course, and 91% resolved by 1 year from initiation (Figure 4A). Among those patients treated with liposomal amphotericin B, median follow-up time was only 4 months (range = 0–6 months); 27 (90%) patients reported improvement, 1 patients reported complete resolution, and 2 patients reported no improvement.

Of 98 PKDL patients who never received treatment, 48 (49%) patients reported resolution, with median time to lesion disappearance of 19 months. Spontaneous resolution was more frequent among children younger than 15 years (37/63 [58.7%]) than patients 15 years or older (11/35 [31.4%]; log-rank \( P = 0.01 \)) (Figure 4B). The rate of spontaneous resolution did not differ by sex, lesion severity, or extent. Maculopapular lesions resolved without treatment more frequently than other types, but this difference was not statistically significant (58% for maculopapular versus 42% for both macular and complex; \( P = 0.22 \)). Patients with spontaneous resolution had a lower frequency of positive results by PCR than those patients whose lesions were unresolved by the end of follow-up, but this difference was not significant (3/19 versus 6/20, \( P = 0.45 \)).

Kala-azar patients had significantly higher serum levels of IFN\( \gamma \), TNF\( \alpha \), and IL-10, whereas IL-12, -13, and -15 and TIMP1 showed less marked but significant elevations compared with controls and patients with cured kala-azar (Figure 5). By contrast, PKDL patients had higher levels of MIP1a, MMP2, and MMP9 compared with kala-azar patients and higher IL-13 and MMP7 than controls (Figure 6). The ratio of MMP9 to TIMP1 (MMP9/TIMP1) was significantly higher in PKDL patients than kala-azar, past PKDL, or past kala-azar patients (median: PKDL = 1.53, kala-azar = 0.326, past PKDL = 0.253, past kala-azar = 0.264; \( P < 0.01 \) for each two-way comparison).

![Figure 3. Photographs of patients with PKDL. (A) Perioral hypopigmented plaques. (B) Perioral hypopigmented macules. (C) Papular lesions on the legs of a child. (D–E) Nodular lesions on the face and arms.](image-url)
Figure 4. (A) Kaplan–Meier survival curve for resolution of PKDL treated with SSG. Estimated resolution rates: 42% within 1 year of onset (6 months after treatment completion), 72% within 2 years, and 92% within 5 years. (B) Kaplan–Meier survival curve for resolution of untreated PKDL. Of 98 untreated PKDL patients, 48 patients had lesion resolution during the follow-up period. Estimated resolution rates: 8% within 1 year of onset, 34% within 2 years, and 67% within 5 years.

Figure 5. Scatter plots showing levels of cytokines among patients with untreated KA, treated cured KA, and untreated active PKDL and healthy North American controls.
Baseline MMP9/TIMP1 was comparable among PKDL patients with subsequent spontaneous resolution and patients who were treated (baseline median = 0.996 versus 0.789, \( P = 0.92 \); post-resolution median = 0.232 versus 0.282, \( P = 0.86 \)). IL-10 and IL-4 levels were higher in complex PKDL compared with macular or maculopapular PKDL (\( P = 0.04 \) for IL-10 in complex versus maculopapular; \( P = 0.005 \) and \( P = 0.03 \) for IL-4 in complex versus macular and all other PKDL). Nevertheless, the distributions by lesion type were largely overlapping (Figure 7). There were no significant associations between anatomical distribution or lesion severity (as defined in Materials and Methods) and any of the serum levels.

**DISCUSSION**

This study provides the most comprehensive picture yet of PKDL in South Asia and highlights the complexity of this phenomenon. Until recently, there were no published PKDL surveillance data from Asia, and cumulative incidence estimates were based on facility-based series. Recent articles suggest that the epidemiology varies widely, even within the Indian subcontinent. Our data showed estimated cumulative PKDL incidence of 3% within 1 year, 10% within 2 years, and 17% within 5 years of kala-azar. By contrast, in a retrospective cohort in Nepal, the incidence was 1.4% within 2 years, 2.5% within 4 years, and 3.6% within 8 years. Our PKDL patients were younger than the patients in the other data, with a median of 12 years compared with 21.5 years in another Bangladeshi study and 24 years in the Nepal study. Young age was the single significant risk factor for PKDL development after kala-azar in our survival analyses as well as the single most significant predictor of resolution without treatment.

Our data showed a similar range of lesion types as previous reports, but in our data, macular and maculopapular forms predominated, whereas facility-based studies report higher percentages of complex forms. In common with earlier reports, the perioral area was often the first affected site, but most patients had extensive lesions by the time of diagnosis. Patients treated for PKDL with SSG showed more rapid resolution than untreated patients, but complete disappearance was slow: only 50% resolved by the end of the 6-month treatment course, and it took 6 more months for an additional 41% to resolve. Our data from the last 8 months of the study suggest that complete PKDL resolution is likely to take more than 6 months, even with liposomal amphotericin. The delay may reflect the process of repigmentation rather than the dynamics of parasite killing. Development of a field-friendly diagnostic test and a sensitive marker for treatment response would greatly facilitate clinical trials for PKDL.

More than one-half of our PKDL patients never received care, and contrary to published dogma, nearly one-half of...
these patients had resolution without treatment. This finding is consistent with data from Sudan, although the proportion was slightly lower in Bangladesh (49%) than Sudan (56%) and time to resolution was longer (median = 19 months versus < 1 year).\textsuperscript{18} Young patients with relatively mild PKDL that resolves without treatment may never be detected without intensive active surveillance. In Sudan, this phenomenon is well-recognized, and treatment is generally withheld unless PKDL is severe or has lasted more than 1 year.\textsuperscript{1} However, these patients may have been previously overlooked in South Asia. Their significance in the anthropootic transmission cycle and the necessity for treatment are two key unanswered questions.

In a study in Nepal, inadequate SSG treatment of kala-azar increased risk of subsequent PKDL.\textsuperscript{17} The high PKDL incidence in our data may be related to the predominant use of SSG; amphotericin seems to provide more rapid immune recovery and may decrease risk.\textsuperscript{19} We were unable to compare PKDL risk by kala-azar treatment drug because of the sparse data for drugs other than SSG in our population. However, PKDL has been reported after all of the major drugs\textsuperscript{20,21} and in patients with no previous antileishmanial treatment. In our data, 10% of PKDL occurred without prior kala-azar, similar to historical data from India.\textsuperscript{22} In contrast to reported SSG resistance in Bihar and Nepal, the SSG failure rate among our kala-azar patients was less than 3%.\textsuperscript{23,24} The high failure rate among patients treated with miltefosine in 2008 was consistent with reports that locally produced miltefosine distributed at that time contained no active ingredient.\textsuperscript{25}

Our cytokine data confirm well-known patterns in kala-azar patients, showing high levels of IL-10, IFN\textgreek{g}, and TNF\textalpha.\textsuperscript{26-28} Our data confirm other studies in showing a mixed profile of inflammatory and anti-inflammatory cytokines. The patient with simultaneous kala-azar and PKDL showed a pattern similar to kala-azar patients. For PKDL without visceral involvement, the systemic cytokine response may be a relatively poor reflection of local skin responses.\textsuperscript{26,29} Studies in affected dermis show mRNA for IFN\textgreek{g}, IL-6 and -10, and TNF\textalpha.\textsuperscript{30} and positive immunohistochemical staining for IFN\textgreek{g} and IL-10 and -4.\textsuperscript{29} With the exception of MIP1\textalpha, we saw few alterations in the serum cytokine profile. However, our data show marked elevations in MMP9 and MMP9/TIMP1 among active PKDL patients, whereas in PKDL patients whose lesions had resolved with or without treatment, MMP9 and MMP9/TIMP1 fell to levels comparable with control groups. MMPs are zinc- and calcium-containing proteolytic enzymes involved in tissue remodeling and leukocyte recruitment, and they are elevated in infectious and non-infectious inflammatory processes.\textsuperscript{30} MMP9 mediates type IV collagen degradation and basement membrane remodeling.\textsuperscript{30} TIMPs are inhibitors of activated MMPs; the ratio of a specific MMP to its TIMP, therefore, reflects the regulatory milieu.\textsuperscript{31} Elevated MMP9 and MMP9/ TIMP occur in tissue and serum from leprosy patients with inflammatory reversal reactions and erythema nodosum leprosum.\textsuperscript{31} PKDL may be associated with a similar removal of TIMP1 inhibition of MMP9, leading to basement membrane degradation, inflammation, and remodeling. MMP9/TIMP1 may provide a serum marker of PKDL and have value for monitoring response to therapy.

Our study had limitations, mostly because of the rural field site and distance from adequate laboratories. Only a small percentage of patients had molecular confirmation, forcing us to rely on clinical characteristics for diagnosis. We were unable to obtain biopsies or peripheral blood mononuclear cells for more extensive immunological assays. Nevertheless, the consistency of the serum findings, especially MMP9/ TIMP1, response to antileishmanial treatment, and coherence of the epidemiological pattern suggest that the clinical diagnoses were accurate in the great majority of cases, including those cases with spontaneous resolution.

PKDL lesions, especially on the face or when widespread, are disturbing to patients, but the real importance of PKDL...
derives from its postulated role as an infection reservoir, especially during interepidemic periods when kala-azar incidence is low. The data on which this assumption relies are diverse. Definitive reservoir confirmation requires demonstration of the ability to infect the vector plus a quantitative assessment of the extent to which this host maintains the pathogen in the natural system.\textsuperscript{32} Infection of laboratory-reared sand flies fed on a patient with nodular PKDL was shown in 1928 followed by positive results in sand flies fed on a patient with nodular PKDL and three patients with macular PKDL in 1933,\textsuperscript{33,34} The only subsequent such experiment was reported in 1992 in four patients with nodular PKDL during an investigation of a kala-azar outbreak; 53% of sand flies showed infection.\textsuperscript{35} One xenodiagnosis-positive patient had migrated from a VL-endemic area and was thought to have introduced the parasite into the outbreak community. As early as the 1930s, Napier and Krisnan\textsuperscript{22} in India observed a common pattern in highly affected communities. Kala-azar incidence rose steeply over 5–10 years and was accompanied by a decrease in average patient age, and then, it fell just as steeply. Simultaneous with the decline in kala-azar incidence, PKDL cases appeared in increasing numbers and eventually predominated over kala-azar. We observed this pattern from 2000 to 2010 in Mymensingh District.\textsuperscript{6,30} A similar but less marked kala-azar incidence peak was seen in 2007 in facility-based data from Bihar, India, although apparently without the PKDL echo.\textsuperscript{37} Napier and Krisnan\textsuperscript{22} attributed the pattern to progressively increasing population-level immunity. Historical records from India, mathematical models, and data on cyclic VL patterns suggest that the epidemic decade just concluded will be followed by a decade of low incidence while the susceptible population accumulates, and without control measures, a new epidemic can be expected 10–15 years from now.\textsuperscript{38–40} Aggressive vector control and treatment programs may alter the course of this cyclic history, but the natural pattern must be taken into account in the interpretation of surveillance data. The Indian subcontinent has been granted an opportunity to recapture the initiative in the VL elimination program. Concentrated efforts, including effective PKDL detection and treatment, are needed now to ensure that the 2000s become the final VL-epidemic decade in South Asia.

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