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Safety and Effectiveness of Short-Course AmBisome in the Treatment of Post–Kala-Azar Dermal Leishmaniasis: A Prospective Cohort Study in Bangladesh

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Background. A safe and effective short-course treatment regimen for post–kala-azar dermal leishmaniasis (PKDL) is considered essential for achieving and sustaining elimination of visceral leishmaniasis (VL) in the Indian subcontinent [1, 2]. Here, single-dose liposomal amphotericin B (AmBisome) has been adopted as a first-line regimen for VL; however the effectiveness and safety of AmBisome for PKDL has not been formally evaluated.

Methods. The safety and effectiveness of AmBisome 15 mg/kg, given over 15 days in 5 biweekly infusions of 3 mg/kg on an outpatient basis, was evaluated between April and November 2014 in patients with clinically diagnosed PKDL, aged ≥12 years and residing in a highly VL-endemic area in Bangladesh. This was a prospective cohort observational study, with the objective to assess final cure 12 months after treatment. Clinical response was monitored at 1, 3, 6, and 12 months, and safety during treatment and up to 1 month after treatment.

Results. Of the 280 patients meeting the inclusion criteria, 273 were assessed at 12 months. A complete or major improvement of lesions was seen in 245 patients (89.7%); 213 (78.0%) were considered completely cured. Lesions did not improve in 28 (10.3%) and new lesions appeared in 13 (4.8%). All patients completed treatment without severe or serious adverse events.

Conclusions. A short-course 15-mg/kg AmBisome regimen proved safe and effective in the treatment of clinically diagnosed PKDL in Bangladesh, and should be considered a treatment option for routine programmatic use in the VL elimination effort in the Indian subcontinent.

Keywords. visceral leishmaniasis; post–kala-azar dermal leishmaniasis; AmBisome; liposomal amphotericin B; Bangladesh; Indian subcontinent.

Post–kala-azar dermal leishmaniasis (PKDL) is a condition in which *Leishmania* parasites persist in the skin after apparently successful treatment of visceral leishmaniasis (VL; kala-azar), probably due to an incomplete immunological response [3]. PKDL occurs in Bangladesh in an estimated 10%–17% of patients, months to years following successful treatment of VL [1, 4, 5].

Individuals with PKDL are usually not ill, and in most, the lesions cause only cosmetic problems. However, persons with untreated PKDL may remain infectious to sand flies for years to decades. PKDL is considered a major challenge to the elimination of VL in the Indian subcontinent [6, 7]. Unlike in Eastern Africa, there is limited evidence that PKDL in Southeast Asia is self-healing [5, 8].

In the Indian subcontinent PKDL cases are treated with miltefosine for 12 weeks, based on a small-scale study that showed cure rates of 93% in 15 patients after 12 months of follow-up [9]. The safety of miltefosine in courses longer than 4 weeks has not been established, and miltefosine's teratogenic effect lasts for >7 months after a 12-week course [10]. It is however rolled out in settings without high compliance to contraception and no continuous availability of pregnancy tests. Compliance to 12 weeks of miltefosine treatment has been shown to be poor [11], and the efficacy of miltefosine in the treatment of both VL and PKDL has been reported to decline in India and Nepal [11–14]. Moreover, miltefosine is difficult and/or expensive to obtain [15].

Médecins Sans Frontières (MSF) aimed to identify an alternative safe, short-course treatment with liposomal amphotericin B (AmBisome; Gilead), based on limited published data that demonstrate its efficacy in PKDL. Initially, a dose of 30 mg/kg was tried, administered in 6 doses of 5 mg/kg over the course of 3 weeks. More than 1400 patients with PKDL were treated with a good outcome; complete recovery of nodular and papular lesions and complete or major repigmentation of macular lesions was observed in 86.5% of patients at 12-month

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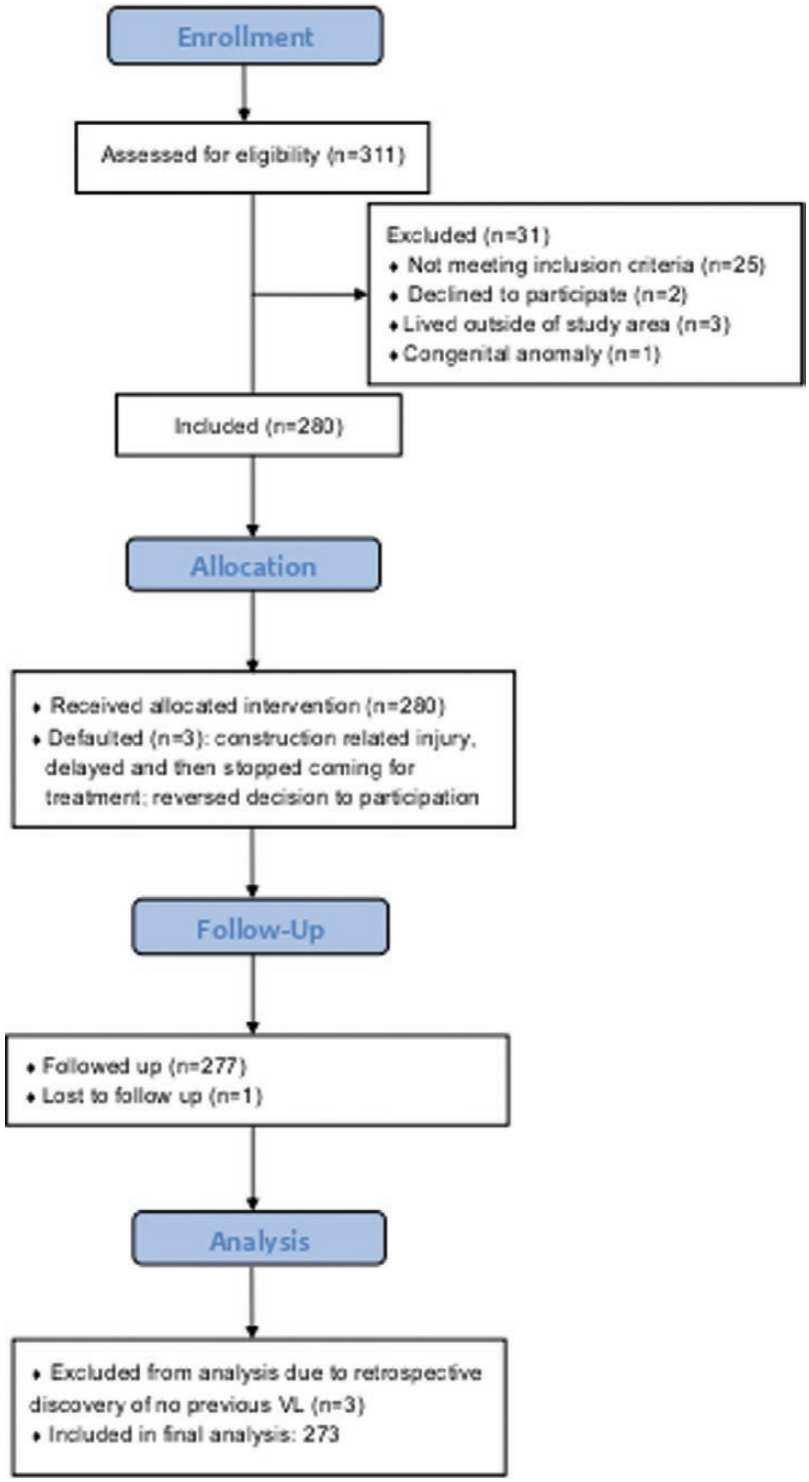

Figure 1. Flow diagram. Abbreviation: VL, visceral leishmaniasis.

follow-up [1, 16]. However, confirmed [6] or possible [17] rhabdomyolysis developed in 25 patients during treatment, probably related to AmBisome-induced hypokalemia [18].

A regimen of 15 mg/kg AmBisome (given over 3 days) was used in >1500 patients with VL, and a regimen of 20 mg/kg AmBisome (given over 4 days) in >12 000 Indian patients with VL without the occurrence of clinical signs related to hypokalemia [19]. Because hypokalemia is known to be a dose-dependent adverse effect of AmBisome, it was assumed that halving its dose could result in a safe regimen for PKDL

Table 1. Patient and Post–Kala-Azar Dermal Leishmaniasis Characteristics

Characteristics	Patients, No. (%) (n = 280)
Age, mean (SD) [median], y	29.5 (15.8) [25.0]
Body mass index, in kg/m^2, mean (SD) [median]	18.6 (2.8) [18.4]
Male/female patients, No.	170/110
Previous VL history (n = 275)	
Time from VL to PKDL, mean (SD) [median], mo	70.1 (53.7) [48]
Previous VL treatment (n = 277)	
SSG	149 (53.8)
AmBisome 15 mg/kg	84 (30.3)
Miltefosine	32 (11.6)
Combination therapy	5 (1.8)
Don't remember[a]	7 (2.5)
Site of lesions	
Face	258 (92.1)
Face and other body parts	215 (76.8)
Type of lesions	
Macular	251 (89.6)
Papular	1 (0.4)
Polymorphic	28 (10.0)
Site where lesions first appeared	
Face	169 (56.8)
Torso	24 (8.6)
Arms	63 (22.5)
Legs	15 (5.4)
Don't know[a]	9 (3.2)
Severity of lesions	
Mild (few lesions, not easy to spot)	18 (6.4)
Moderate (easy to spot, plenty of normal skin)	196 (70.0)
Severe (densely covered with lesions, hardly any normal skin to be seen)	66 (23.6)

Abbreviations: PKDL, post–kala-azar dermal leishmaniasis; SD, standard deviation; SSG, sodium stibogluconate; VL, visceral leishmaniasis.

Data represent No. (%) of patients unless otherwise specified.

[a]Refers to patient self report.

Table 2. Post–Kala-Azar Dermal Leishmaniasis Presentation

Feature	Patients, No. (%)
Time since VL treatment (n = 275)	
Mean (SD) [median], mo	70.1 (53.7) [48]
Range, y	0–30
<1 y	1 (0.4)
1–4 y	142 (51.6)
5–9 y	84 (30.5)
10–14 y	30 (10.9)
≥15 y	18 (6.5)
Duration of PKDL lesions (n-= 260)	
Mean (SD) [median], mo	30.2 (34.8) [18]
Range, mo	1–192
1–3 mo	46 (17.7)
4–6 mo	31 (11.9)
≥1 y	172 (66.2)
≥3 y	86 (33.0)
≥5 y	47 (18.0)
≥10 y	9 (3.5)

Abbreviations: PKDL, post–kala-azar dermal leishmaniasis; SD, standard deviation; VL, visceral leishmaniasis.

Data represent No. (%) of patients unless otherwise specified.

that would still be effective. As an extra measure of care, the total dose was divided into 5 doses of 3 mg/kg given over 15 days. In this article, we report the safety and efficacy of 15 mg/kg AmBisome treatment for PKDL, given over 15 days in 5 biweekly infusions of 3 mg/kg, as an ambulatory treatment in a primary healthcare setting.

METHODS

Trial Design and Patients

This was a prospective cohort study conducted in the MSF leishmaniasis clinic in Fulbaria upazila (subdistrict), Mymensingh district, Bangladesh. Patients aged ≥12 years were recruited via active case finding and self-referral from Fulbaria, Trishal, Modhupur, Muktagaccha, Ghatail, and Gaffargaon upazilas. Diagnosis of PKDL followed the World Health Organization (WHO) recommendations for PKDL diagnosis in the field setting (skin lesions strongly suggestive of PKDL after visual examination, a history of VL treatment, and a positive serological rK39 test result) [17]. Enrolled patients were admitted and treated after giving informed consent for both the treatment and use of photography to evaluate outcomes.

A study team of 3 physicians was trained to visually recognize PKDL lesions with the aid of the WHO *Post Kala-Azar Dermal Leishmaniasis (PKDL) Atlas* [20]. Cases with ambiguous lesions were advised to return to the clinic after 2 weeks, offered treatment with antifungal medication, or referred to a dermatologist. Patients with PKDL and concurrent VL, those who had received prior treatment for PKDL, those taking medication with an adverse effect profile overlapping that of AmBisome, and those with a known hypersensitivity to AmBisome were excluded. Pregnancy and breastfeeding, preexisting cardiac disease, hepatic impairment, or other severe chronic underlying diseases were also reasons for exclusion.

A potassium monitoring and supplementation safety protocol was developed for the study, wherein patients with renal impairment (baseline serum creatinine level >1.3 mg/dL) were excluded and those with baseline serum potassium levels <3.5 mmol/L were included only after serum potassium had been corrected to at least one reading with a level >4.2 mmol/L. Patients with diarrhea, vomiting, or other conditions resulting in significant body potassium loss at the time of enrolment were asked to return for PKDL treatment once recovered.

Treatment

At baseline, medical photographs of each body part affected by lesions were taken in a standardized manner (face: front and lateral; torso: prone, supine, and lateral; arms and legs: prone, supine, lateral, and medial). If normal baseline values of potassium and creatinine were obtained, a test dose of 1 mg of AmBisome was administered over 10 minutes, after

Table 3. Treatment Outcomes at 12 Months

Outcome	Patients, No. (%)
Descriptive categories (n = 273)	
Category 1: Complete resolution of nodular and papular lesions, and complete or almost complete repigmentation of macular lesions	213 (78.0)
Category 2: Complete resolution of nodular and papular lesions and major repigmentation of macular lesions; some resolution of macular lesions	32 (11.7)
Category 3: No or limited improvement of lesions, but no new lesions	15 (5.5)
Category 4: No or limited improvement of lesions with emergence of new lesions	13 (4.8)
Weighed percentage of improvement in patients responding to therapy (n = 258)[a]	
Mean improvement (SD) [median]	85 (14.6) [90]
≥50% improvement	249 (96.5)
≥80% improvement	208 (80.6)
≥90% improvement	131 (50.8)

Data represent No. (%) of patients unless otherwise specified.
Abbreviation: SD, standard deviation.

[a]For 1 patient, it was not possible to produce a weighed score owing to difficulties in interpreting photographs.

which the patient was observed carefully for 30 minutes for a hypersensitivity reaction. Patients then received a total AmBisome dose of 15 mg/kg, given in 5 doses of 3 mg/kg, infused over 2 hours in 5% dextrose solution in a biweekly dosing schedule. Patients were monitored for serum potassium, magnesium, and creatinine before administration of the first, third, and fifth (last) dose and 1 week after the last dose. Serum potassium and creatinine measurements were repeated 1 month after the last dose if hypokalemia and/or elevated creatinine were present 1 week after treatment.

The potassium supplementation safety protocol prescribed the following: If the serum potassium level is <4.2 mmol/L, start oral potassium and magnesium supplementation; if the serum potassium level is <3 mmol/L, test serum creatine phosphokinase and if this value is >1000 U/L and myoglobinuria is present, admit the patient for continuous intravenous fluids plus oral potassium and magnesium supplementation; if the serum potassium level is <2.8 mmol/L, stop AmBisome treatment, and do not resume until serum potassium is corrected with intravenous supplementation and refer the patient for an electrocardiogram. Patients were kept under observation for a minimum of 3 hours after starting treatment and discharged only if they were generally well, ambulant, and not showing symptoms associated with hypokalemia. All were instructed to call a dedicated 24/7 emergency telephone number and return to the clinic as soon as possible if they felt unwell or experienced abnormal symptoms.

Outcomes

All patients were requested to come for follow-up visits at 1 week and 1, 3, 6, and 12 months after completion of the last dose and were reimbursed for transport costs for any visit to the clinic. All efforts were made by the outreach teams to encourage patients to attend their follow-up appointments. Loss to follow-up at 12 months was defined as any patient not reporting between 1.5 month before and 3 months after the scheduled date.

Lesions were photographed during the follow-up visits, and their evolution was scored as percentage improvement compared with baseline by each of the study team's physicians, who were blinded to each other's assessments. The score was weighed; at baseline, a percentage was assigned for the relative measure in which each affected body part (face, torso, arms, and legs) contributed to the total burden of lesions. If the weighed scores by all 3 physicians did not differ more than 20%, an average score was used, but if a wider degree of variation was observed the photographs were reanalyzed and an agreed-on score was assigned.

For each body part, the relative improvement was calculated as follows: relative improvement = (contribution at baseline [%] × improvement [%])/100. The overall improvement was calculated as the sum of the relative improvement for each body part. The final outcome at 12 months was also scored using descriptive categories after careful evaluation of all photographs of each patient by each of the study physicians, who were blinded to each other's assessments. The photographs were then evaluated by a fourth expert to ensure consistency of scoring.

Patients in category 3 or 4 were referred to Ministry of Health facilities for further assessment and possible treatment with miltefosine. Polymerase chain reaction (PCR) testing to confirm *Leishmania* infection was conducted by the International Centre for Diarrhoeal Disease Research, Bangladesh in Dhaka using established techniques [21]. The assessment of safety during treatment and follow-up was based on clinical adverse events, laboratory parameters during treatment, and a clinical assessment during the first 2 follow-up visits (1 week and 1 month after treatment).

Sample Size

Assuming that the 15-mg/kg AmBisome regimen will be only slightly less effective than the 30-mg/kg regimen, for an estimated effectiveness of minimally 80%, with a precision of 5% and a significance level of 5%, a sample size of 250 patients was needed. Assuming a loss to follow-up of 10%, the total sample size was calculated as 275 patients.

Ethical Approval

Ethical clearance was obtained from the Bangladesh Medical Research Council and the MSF Ethical Review Board. The study was designed in accordance with the general ethical principles outlined in the Declaration of Helsinki and the International Committee for Harmonization guidelines for Good Clinical

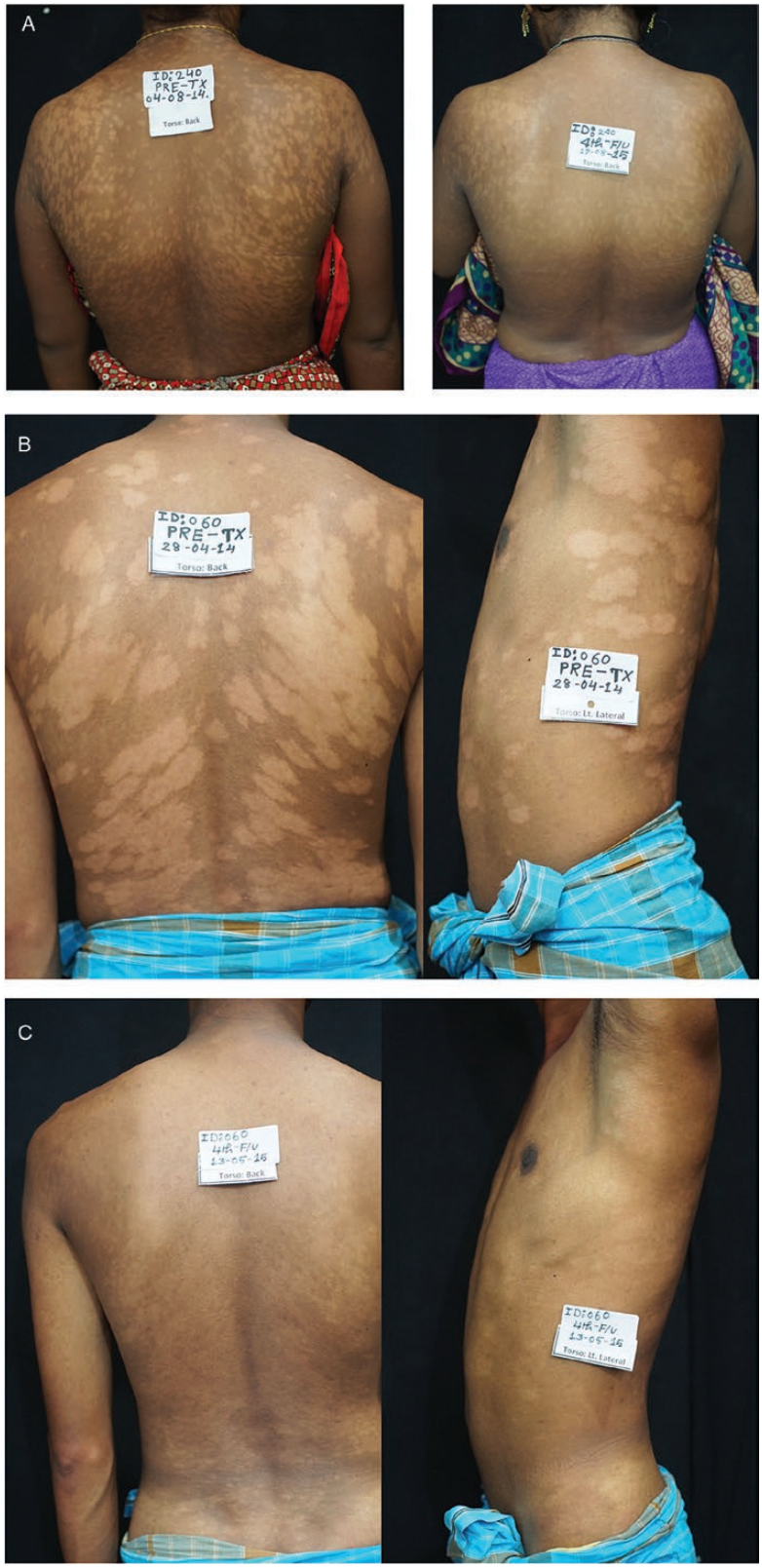

Figure 2. (*A-C*) Patients with marked and extensive macular lesions. A good treatment response was observed, but full repigmentation was not yet achieved at 12 months.

Practice, and complied with all applicable state, local and foreign laws protecting the rights and welfare of human subjects. The study was registered retrospectively at clinicaltrials.gov under number NCT03311607.

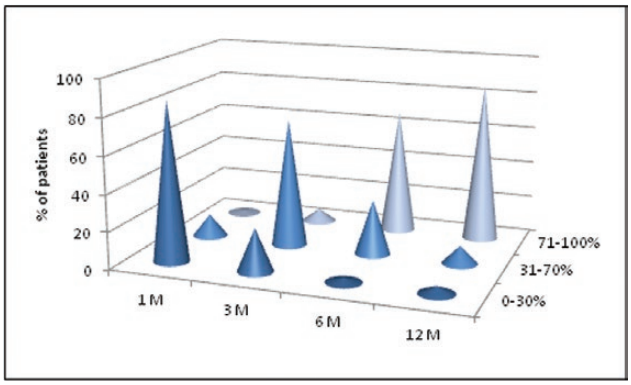

Figure 3. Improvement of lesions over 12 months.

RESULTS

Patients

Of 311 patients with a clinical diagnosis of PKDL, 280 were eligible for inclusion in the study. The most common reasons for exclusion were severe chronic underlying disease or renal impairment (4 patients), previous PKDL treatment (7 patients), and age <12 years (5 patients). The patient flow through the study is shown in Figure 1. Patients were recruited between 8 April and 25 November 2014, and follow-up of all patients was completed in December 2015.

Baseline characteristics are given in Table 1. The majority of patients (89.6%) presented with macular lesions. All but 4 patients indicated that they had completed their VL treatment, and 18 (6.5%) had experienced a VL relapse before presenting with PKDL. The median time between the VL episode and presentation at the clinic was 48 months (Table 2). The delay in PKDL treatment seeking was considerable, with a median time of 18 months, suggesting that the median time for development of PKDL symptoms after VL treatment is 30 months, similar to earlier findings by MSF (36 months, based on data obtained from >1100 patients with PKDL; data presented at MSF's Scientific Day in London, 2012) [22].

Response to Treatment

Response to treatment at 12 months is shown in descriptive categories, as well as in weighed percentages (Table 3). Complete resolution of nodular and papular lesions, and complete or almost-complete repigmentation of macular lesions had occurred in 213 patients (78.0%) (category 1); in another 32 (11.7%), major improvement of macular lesions was seen (category 2) (Figure 2A-C). The weighed percentage outcomes at 12 months corresponded with these results; 80.6% of patients for whom weighed percentages could be calculated (n = 258; see Table 3) showed an improvement of ≥80%. The improvement of lesions at 1, 3, 6, and 12 months in weighed percentages as compared with baseline is shown in Figure 3. In the majority of patients, response to treatment started after 1 month, but resolution of lesions (71%–100% improvement) is not seen until 6 months after treatment.

No or limited improvement of lesions (with no new lesions) was observed in 15 patients (5.5%). Four had extensive macular lesions that gradually worsened; skin biopsy–PCR tests could be performed in 3 patients after 12 months, and these results were positive for *Leishmania* infection. In 13 (4.8%) of patients new lesions appeared, in 7 cases after initial improvement (Figure 4). One patient had irregular pigmentation on the chest that was unresponsive to treatment; a skin biopsy–PCR

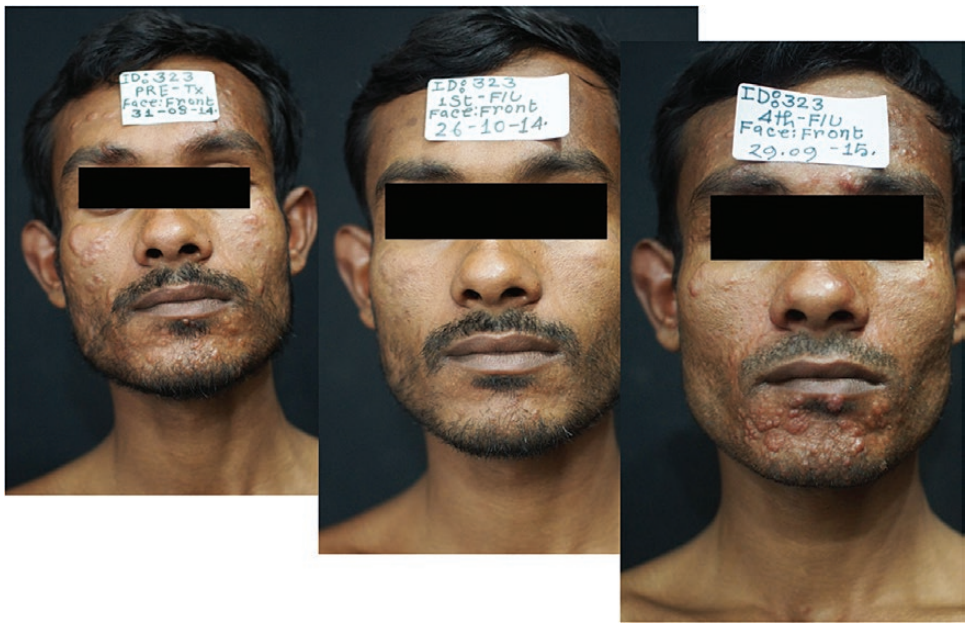

Figure 4. Recurrence of papular lesions at 12 months.

Table 4. Laboratory Values During and 1 Week After Treatment (n = 280)

Abnormal Laboratory Values	Patients, No. (%)
Hypokalemia (serum potassium range)	
Mild (3.0–3.5 mmol/L)	75 (26.8)
Moderate (2.5–2.99 mmol/L)	7 (2.5)
Severe (<2.5 mmol/L)	0 (0)
Serum creatinine >1.3 mg/dL	8 (2.9)

test was performed 1.5 year after treatment, with negative results. It was concluded that this patient's condition was initially misdiagnosed.

Safety

There were no severe or serious adverse effects reported during the study. Serum potassium levels were below normal in 82 patients (29.3%) (Table 4 and Figure 5) but returned to normal within 1 week of potassium supplementation. Two patients complained of general weakness, but all others remained asymptomatic, and creatine phosphokinase levels and electrocardiographic recordings remained normal. Creatinine, if raised, returned to normal values within 4 weeks, with one exception (at 12 weeks).

A total of 214 patients (76.4%) experienced at least one adverse event; these were mostly mild. General weakness, anorexia, vertigo, nausea, and pyrexia were most frequently reported among the infusion-related adverse effects (in >10% of patients; Table 5).

DISCUSSION

We observed a good treatment response to the 15-mg/kg AmBisome regimen, with 89.7% of patients showing complete resolution of papular lesions and major to complete repigmentation of macular lesions. Safety was acceptable, with no serious adverse events. If the serum potassium level was low during treatment, it returned to normal within 1 week. We conclude that this regimen can be used without serum potassium monitoring. Both physicians administering AmBisome and patients, however, should be aware of the signs and symptoms of severe hypokalemia.

The repigmentation of macular lesions depends on the regeneration of damaged melanocytes and renewed melanogenesis [23]. In our study, macular lesions took several months to resolve, an observation also made in other studies and in a cohort of patients with previously treated PKDL (MSF; under publication) [24, 25]. Extended treatment courses, lasting several months, have for this reason been the conventional approach for PKDL in the Indian subcontinent [1]. We hypothesize that short courses are equally effective: drug treatment brings the parasite load down substantially, after which an immunological response occurs and clinical cure follows. It should be noted that antileishmanial drugs also influence the immune response, including AmBisome [3]. Seeing that decreasing the total dose of AmBisome from 30 to 15 mg/kg did not lead to decreased effectiveness in curing PKDL lesions, we propose that even shorter courses and lower doses of AmBisome may be effective in PKDL. AmBisome is available free of charge for PKDL until 2021 [26], which offers an opportunity to explore this and and to conduct additional observational randomized clinical trials. PKDL can present in extreme and very severe nodular forms, particularly in India, not encountered in this study; for such patients, prolonged and more intensive treatment may be needed

The diagnosis and evaluation of PKDL lesions via medical photographs was externally validated by experts in earlier data sets (MSF, under publication). In our opinion, the descriptive categories that we chose are useful for field purposes in defining treatment outcome at 12 months when more complex assessments are not feasible. There was little inter- and intraobserver variability between the different assessors, and outcomes corresponded to our detailed quantitative assessment of the improvement of lesions. In another PKDL study performed in India, the clinical outcome was assessed in a similar manner [12].

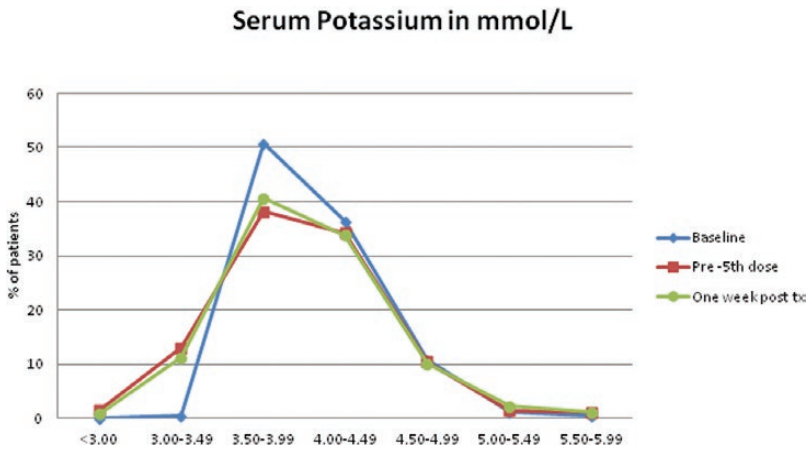

Figure 5. Serum potassium levels.

Table 5. Most Common Adverse Events Definitely, Probably, or Possibly Related to Treatment (Intent-to-Treat Population, n = 278)

Adverse Events[a]	Patients, No. (%)
Patients with ≥1 adverse event	214 (77)
Serious adverse events recorded	0 (0)
Treatment discontinued because of adverse events (related or unrelated)	0 (0)
Abdominal pain	27 (10)
Bitter taste in mouth	5 (2)
Body aches	8 (3)
Burning sensation	3 (1)
Cough	5 (2)
Dental infection	5 (2)
Dyspepsia	18 (6)
Heaviness of head	13 (5)
Oral infection	4 (1)
Lower back pain	3 (1)
Muscle pain	11 (4)
Muscle weakness	4 (1)
Neck pain	10 (4)
Runny nose	8 (3)
Urinary tract infection	4 (1)
General weakness	60 (22)
Anorexia	58 (21)
Vertigo	60 (22)
Pyrexia	30 (11)
Nausea	43 (16)
Vomiting	26 (9)
Headache	34 (12)
Skin allergy	22 (8)
Diarrhea	29 (10)
Any back pain	17 (6)
Chest tightness	12 (4)
Other effects	22 (8)

[a]Adverse events occurring in >1% of patients.

We observed 7 relapses (2.6%) after initial improvement, all occurring within 6–12 months after treatment. PKDL relapses may not be drug or dose related; the probable culprit may be an impossibility to achieve parasitological cure, combined with an individual genetic predisposition for developing chronic PKDL or an immune response that varies over time [1, 3]. More relapses may have occurred after 12 months; in 73 Indian patients with PKDL treated with miltefosine, relapse occurred in 4% (3 relapses) within 12 months, but this rate increased to 15% (11 relapses) after 18 months; in 18 patients treated with miltefosine, 2 relapsed after 17 months, and 1 after 39 months [11, 25]. It is concerning that parasite isolates of these relapses showed a decreased sensitivity for miltefosine [25]. The efficacy of 12 weeks of miltefosine has since been questioned, and 16-week courses may be necessary [11].

We found that the median treatment seeking delay for PKDL was 18 months, and that a third (33%) of patients had lesions for more than 3 years before they received treatment. The majority (97.5%) of our patients were recruited via active case finding; with reliance on passive reporting, treatment delays are likely to be even longer [1]. Seeing the demonstrated infectivity of macular PKDL lesions in Bangladesh [27], active case finding, as now practiced by the Ministry of Health in Bangladesh and India, is essential to target the remaining PKDL burden, especially now that the elimination target of an incidence of VL cases <1 per 10 000 persons at the subdistrict level is close to being reached in Bangladesh [2, 16]. The here-described AmBisome 15 mg/kg regimen may be the best option available for patients with PKDL in the Indian subcontinent today and can serve as a safe, effective, and feasible alternative to 12 weeks of miltefosine.

Our study had some limitations. PKDL in Bangladesh presents in a variety of forms, from very small to large polymorphic or macular lesions, which can be spread over the entire body or affect only a single area. There are multiple diseases that present with similar lesions, in particular, fungal infections such as pityriasis versicolor. Parasitological assessment was not done, because the sensitivity of microscopic detection of *Leishmania* parasites in skin biopsy and skin slit specimens is limited, varying between 67% and 100% in nodular but only 3%–33% in macular lesions, while macular PKDL is by far the predominant form, seen in >95% of patients in Bangladesh. In the current study, we retrospectively demonstrated in one patient that PKDL had probably been the wrong diagnosis. PCR may increase diagnostic sensitivity, but it has not been validated as a test of cure [20, 28, 29]. The study team, when in doubt, initially prescribed miconazole cream, an approach we recommend for rollout in a primary health care setting, as well as the distribution of visual aids, such as the WHO *Post Kala-Azar Dermal Leishmaniasis (PKDL) Atlas* [20] and the WHO self-learning course on PKDL [22].

Notes

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Potential conflicts of interest. All authors: No reported conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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