Am. J. Trop. Med. Hyg., 96(2), 2017, pp. 285–291
doi:10.4269/ajtmh.16-0432

Protective Efficacy of Secondary Prophylaxis against Visceral Leishmaniasis in Human Immunodeficiency Virus Coinfected Patients over the Past 10 Years in Eastern India

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Abstract. Coinfection with visceral leishmaniasis (VL) and human immunodeficiency virus (HIV) leads to frequent treatment failure, relapse, and death. In this retrospective analysis from eastern India (2005–2015), our primary objective was to ascertain the protective efficacy of secondary prophylaxis with monthly amphotericin B (AmB) given in patients with HIV–VL coinfection toward reducing relapse and mortality rates. The secondary objective was to compare clinical features, laboratory findings, and treatment outcomes in HIV–VL patients in contrast to VL monoinfection. Overall, 53 cases of HIV–VL and 460 cases of VL monoinfection were identified after excluding incomplete records. Initial cure rate was 96.23% in HIV–VL (27 received liposomal AmB and 26 AmB deoxycholate). All patients with initial cure ($N = 51$) were given antiretroviral therapy. Secondary prophylaxis ($N = 27$) was provided with monthly 1 mg/kg AmB (15 liposomal, 12 deoxycholate). No relapse or death was noted within 6 months in the secondary prophylaxis group (relapse: none versus 18/24 [75%]; mortality: none versus 11/24 [45.8%]; $P < 0.001$ for both). Secondary prophylaxis remained the sole significant predictor against death in multivariate Cox regression model (hazard ratio = 0.09 [95% confidence interval = 0.03–0.31]; $P < 0.001$). HIV–VL patients had higher 6-month relapse rate, less relapse-free 12-month survival, and higher mortality ($P < 0.001$ each) than VL monoinfection. In conclusion, it appears from this study that secondary prophylaxis with monthly AmB might be effective in preventing relapse and mortality in HIV–VL.

INTRODUCTION

Visceral leishmaniasis (VL) is endemic in India, a country that also contributes maximally to the global burden of the disease.[1–3] India also ranks third as a country with gross number of people living with human immunodeficiency virus (HIV)/acquired immunodeficiency syndrome (AIDS), harboring almost 6% of the world population of HIV-infected people, with a prevalence of HIV around 0.27%.[4,5]

The problem of HIV and VL (HIV–VL) coinfection is recently being recognized as a major hurdle for disease control with significant public health implications: *Leishmania* has evolved strategies to survive and multiply within macrophages in HIV patients,[6] engendering the possibility of drug resistance and treatment failure.[7,8] The risk of developing VL is 100–2,300 times higher in HIV-infected patients than in HIV-negative individuals.[9,10] Though randomized controlled trials (RCTs) are yet to be available in the arena of HIV–VL, it is generally accepted that worse outcome, higher relapse rate, mortality, drug toxicity, and resistance are common in this group of patients.[10,11] Lack of RCT and high-quality evidence from well-designed studies in the field of HIV–VL coinfection makes treatment decisions and prognostications difficult.

In this retrospective analysis from eastern India (2005–2015), our primary objective was to ascertain the protective efficacy of secondary prophylaxis with monthly amphotericin B (AmB) given in patients with HIV–VL toward reducing relapse and mortality rate. The secondary objective was to compare clinical features, laboratory findings, and initial treatment outcomes in HIV–VL in comparison with VL monoinfection.

MATERIALS AND METHODS

This retrospective observational study was conducted at the School of Tropical Medicine (STM), Kolkata, India, a tertiary care referral center for both VL and HIV cases for over 20 years. All patients admitted at the STM from January 2005 to February 2015 with VL were retrospectively included. Investigators' clinical records of patients managed under their care at inpatient and outpatient departments were analyzed anonymously. Hospital records of routinely collected data generated during inpatient management were analyzed with permission from the appropriate authorities. The hospital records that are stored as hard copies in the records department under supervision of the registrar of STM were sorted yearwise. Case records which showed an admission linked to the diagnosis or treatment of VL were extracted. These were further sorted based on availability of clear mention of the components of "case definition" for VL (vide infra). Approval of the Clinical Research Ethics Committee (CREC) of the STM was sought and obtained to undertake this study (CREC approval no. CREC-STM/307, date: January 9, 2016).

Diagnosis of Visceral Leishmaniasis. Case definition of VL (all three were needed for inclusion)

1. Symptoms and signs suggestive of VL (fever for more than 2 weeks and splenomegaly, from an endemic area)
2. rK39 immunochromatographic strip test (ICT) (Kalazar Detect™; InBios International Inc., Seattle, WA) positivity
3. Demonstrable amastigotes of *Leishmania* parasites (Leishman-Donovan bodies) in splenic or bone marrow aspirates.

Method of HIV diagnosis. HIV testing at STM was performed with three parallel rapid diagnostic tests (CombAIDS RS

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Advantage ST, Span Diagnostics Pvt. Ltd., Udhna, Surat, Gujarat, India; Tri-Line [Pareekshak], Bhat Biotech Pvt. Ltd., Bangalore, Karnataka, India; and SD Bioline, Bio Standard Diagnostics Pvt. Ltd., Manesar, Gurgaon, Haryana, India) with positive results in all three required for diagnosis of HIV positivity. Discordant results were confirmed with Western Blot. Only records that clearly indicated HIV testing status were included for the study. HIV testing is undertaken on the basis of provider-initiated counseling and testing or direct walk-in client policy in our hospital.

Treatment and follow-up. The initial therapy for both VL monoinfection and HIV–VL coinfection were recorded. Primary outcome was the rate of VL relapses within 6 months of treatment. Secondary outcome measures were relapse-free 12-month survival and mortality during follow-up. Initial cure was defined as clinical and parasitological cure within 30 days of treatment initiation. Clinical cure was defined as absence of fever and any other constitutional symptoms along with regression of splenomegaly ($\geq$ 50%) and improvement of hematological parameters (hemoglobin $\geq$ 10 gm/dL) within 30 days of follow-up. To assess parasitological cure, splenic aspirate (or bone marrow aspirate in whom spleen was not palpable) was performed on or before day 30 after therapy initiation. Relapse was defined as reappearance of signs or symptoms suggestive of leishmaniasis after initial cure, followed by identification of LD bodies in splenic aspirate (or bone marrow aspirate if spleen is not palpable or less than 5 cm palpable) within or after 6 months. Relapse-free 12-month survival was defined as hospital records showing regular 12 months of follow-up without any suggestion of relapse or requirement of retreatment for VL after initial cure. Treatments offered for relapses of VL and deaths due to VL at STM after initial cure were noted.

We usually assess suspected and confirmed VL cases with serum and urine rK39 ICTs. All hospital records with rK39 positivity were noted and analyzed.

Secondary prophylaxis. Records of HIV–VL patients who were offered secondary prophylaxis were documented in terms of drugs and doses used and the duration of secondary prophylaxis.

Exclusion criteria. The records with following deficits were excluded: any record not fulfilling any one of the three criteria for diagnosis of VL, records which did not clearly indicate whether testing for HIV was undertaken, records which did not provide data regarding CD4 cell count at baseline or at 6 months of follow-up of HIV–VL cases, and records of those patients lost to follow-up within 12 months of initial cure. Those patients who were lost to follow-up or could not be contacted through telephone were also excluded.

The following data were recorded: age, gender, occurrence, and number of relapses of VL previously; clinical symptoms and signs pertaining to VL; HIV-positive or negative status, tuberculosis coinfection at any point of time after initial entry in STM records; hemoglobin (gm/dL), total leukocyte count (/μL), and platelet count (/μL) at baseline only; CD4 cell count (/μL) at baseline and at 6 months of follow-up after initial cure in HIV–VL coinfected patients; initial cure rate, relapses within first 6 months of follow-up after initial cure, and relapse-free 12-month survival after initial cure, and mortality rate.

Statistical methods. Continuous data were presented as mean ± SD and categorical data as number (proportion). Comparison of means was tested with Student's *t* test or Mann–Whitney *U* test and comparison of proportions were undertaken with χ^2 test or Fisher's exact test, as was deemed appropriate.

Three separate analyses were done. First was comparison of clinical features, diagnostic and treatment outcome of patients with VL monoinfections versus those with HIV–VL coinfections. Second was treatment outcome of patients with HIV–VL coinfection within 12 months of follow-up after initial cure. And the last was survival analysis of patients with HIV–VL with death as dependent variable. In survival analysis, variables associated with increased mortality from univariate analysis with $P < 0.2$ were selected for entry into multivariate Cox regression model, taking into account effects of several potential factors simultaneously and then hazard ratios (HRs) were obtained. All statistical analyses were done using SPSS, version 16 (SPSS Inc., Chicago, IL).

RESULTS

Records of 53 patients with HIV–VL coinfection were included (Figure 1) with 41 males (77%, 95% confidence interval [CI] = 64.5–86.6) and mean age 34 ± 8.8 years (median = 34 years, interquartile range = 12 years). Mean CD4 count at presentation was 98.1 ± 58.6 /μL. Among these patients, 22 (41%, 95% CI = 29.3–54.9) had prior documented VL infections. Patients who presented with relapses had a mean of 1.5 relapses of VL before the diagnosis of HIV.

Clinical features of HIV–VL coinfection. The clinical features of HIV–VL coinfection were fever (100%), weight loss (98%), pallor (75%), hepatomegaly (91%), and splenomegaly (100%). Fifteen (28.30%) patients with HIV–VL presented with atypical clinical features—gastrointestinal in seven (13.2%), respiratory in six (11.3%), and bleeding in two (3.8%) (Table 1).

Comparison of clinical features, laboratory diagnostics, and treatment outcomes between patients with VL monoinfection and HIV–VL coinfection. We accessed 460 VL-monoinfected patients' case records with complete information. The mean age was 28 ± 14 years with 225 males (48%, 95% CI = 44.4–53.5). Patients with HIV–VL had significantly lower level of hemoglobin compared with monoinfected patients. When tested with serum samples, rK39 ICTs were positive in 50 (94.3%, 95% CI = 84.6–98.1) coinfected compared with 454 (98.7%, 95% CI = 97.2–99.4) monoinfected VL patients, with trend toward lower serum sample positivity in HIV–VL ($P = 0.056$). However, results of rK39 ICT positivity with urine samples in HIV–VL coinfected patients were similar to monoinfected patients (98.1%, 95% CI = 90.0–99.7 versus 99.4%, 95% CI = 98.1–99.8; $P = 0.33$). Initial cure rates were comparable in both groups (HIV–VL versus VL, respectively, 51/53 [96.2%, 95% CI = 87.3–98.9] versus 456/460 [99.1%, 95% CI = 97.8–99.7], $P = 0.12$) with same regimen—AmB deoxycholate (1 mg/kg for alternate days up to a total dose of 20 mg/kg) (HIV–VL: 26 patients, VL monoinfection: 335 patients) or liposomal AmB (7.5 mg/kg on two consecutive days for a total 15 mg/kg),[12] (HIV–VL: 27 patients, VL monoinfection: 125 patients). Coinfected patients with initial cure had significantly more relapses within first 6 months of

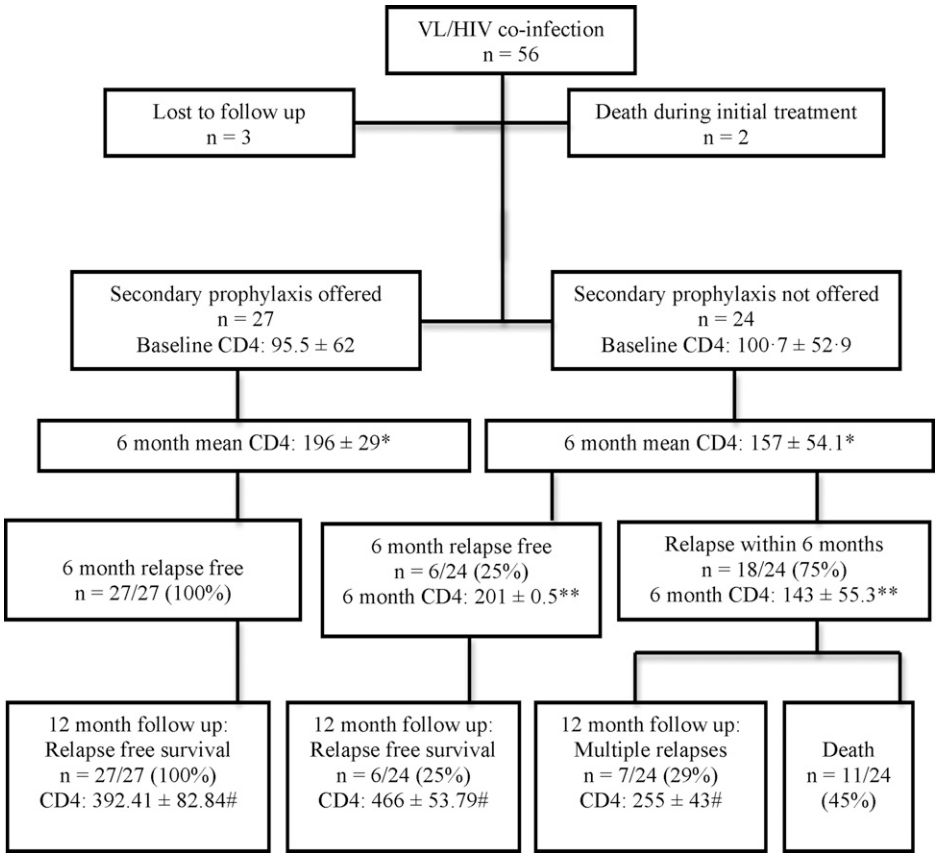

FIGURE 1. Baseline and follow-up data of HIV–VL coinfected patients. * Comparison of mean CD4 counts at 6 months between the groups of patients who did or did not receive secondary prophylaxis; *P* value (Mann–Whitney *U* test) = 0.015. ** Comparison of mean CD4 counts at 6 months within the group of patients who did not receive secondary prophylaxis, between the patients who did or did not experience relapses within the first 6 months of follow-up; *P* value (Student's *t* test) of = 0.007. # Comparison of 12-month mean CD4 counts among the surviving patients; *P* value (analysis of variance) ≤ 0.001, Tukey's post hoc all *P* values are < 0.001 except post hoc comparison between the patients who received secondary prophylaxis vs. patients with a 12-month relapse-free survival despite not having secondary prophylaxis (*P* = 0.085). HIV–VL = human immunodeficiency virus–visceral leishmaniasis.

follow-up (18/51 [35.3%, 95% CI = 3.6–49] versus 30/456 [6.6%, 95% CI = 4.7–9.2], respectively, $P < 0.001$) and significantly less relapse-free 12-month survival (33/51 [64.7%, 95% CI = 50.9–76.4] versus 418/456 [91.7%, 95% CI = 88.8–93.9], respectively, $P < 0.001$). Mortality rate of patients with HIV–VL coinfection (11/51, 21.6%, 95% CI = 12.5–34.6) was significantly higher than VL monoinfected patients (2/456, 0.4%, 95% CI = 0.12–1.6) with $P < 0.001$ (Table 2).

TABLE 1
Distribution of typical and atypical clinical symptoms and signs in HIV–VL coinfected patients

Symptoms and signs		*N* (%)	95% confidence interval
Fever		53 (100)	93.24–100
Weight loss		52 (98)	90.06–99.67
Pallor		40 (75)	62.43–85.07
Hepatomegaly		48 (91)	79.75–95.9
Splenomegaly		53 (100)	93.24–100
Atypical features		15 (28)	17.97–41.57
Distribution of atypical features	Diarrhea	7 (13)	6.55–24.84
	Cough	6 (11)	5.29–22.58
	Shortness of breath	2 (4)	1.04–12.75
	Menorrhagia	2 (4)	1.04–12.75
	Per-rectal bleeding	1 (2)	0.33–9.94

HIV–VL = human immunodeficiency virus–visceral leishmaniasis.

Treatment and secondary prophylaxis in patients with HIV–VL coinfection (Figure 1). Coinfected patients were treated with AmB deoxycholate or liposomal AmB. Two patients who presented with two previous relapses did not respond to initial treatment with AmB deoxycholate and died in hospital. All patients were given antiretroviral treatment (ART) (fixed drug combination containing tenofovir 300 mg + lamivudine 300 mg + efavirenz 600 mg daily) following AmB total dose. Mean time to start ART from study entry in patients receiving AmB deoxycholate was 33 ± 10 days and 13 ± 3 days for patients receiving liposomal AmB .

Twenty-seven patients (52.9%, 95% CI = 39.5–65.9) received secondary prophylaxis (15 patients with intravenous liposomal AmB 1 mg/kg monthly and 12 patients with intravenous AmB deoxycholate 1 mg/kg monthly). Secondary prophylaxis was stopped after 6 months in 25 patients when CD4 count rose above 200 /μL. It had to be continued beyond 6 months in two patients (till 7th and 8th months, respectively) till CD4 rose above 200 /μL. There was no statistically significant difference between baseline CD4 counts in patients with or without secondary prophylaxis

TABLE 2
Comparison of demographics, clinical, diagnostic, and therapy-related results in patients with HIV–VL vs. VL monoinfection

Variables at baseline	Parameters	VL *N* = 460	HIV–VL *N* = 53	*P* value
Age in years	Mean ± SD	28 ± 14	34 ± 8	0.67
Male gender	*N* (%)	225 (48.9)	41 (77.4)	< 0.001
Liver palpable below right costal margin (in cm)	Mean ± SD	3.4 ± 2.1	3.9 ± 1.8	0.41
Spleen palpable below left costal margin (in cm)	Mean ± SD	9.4 ± 3.9	9.7 ± 3.3	0.37
Hemoglobin at baseline (gm/dL)	Mean ± SD	7.8 ± 1.4	7.5 ± 1.7	0.01
Total leukocyte count (/μL)	Mean ± SD	2,457 ± 1,905	2,722 ± 1,486	0.11
Platelet count at baseline (/μL)	Mean ± SD	1.3 ± 0.5	1.3 ± 0.7	0.71
rK39 positivity in serum	*N* (%)	454 (98.7)	50 (94.3)	0.056*
rK39 positivity in urine	*N* (%)	457 (99.3)	52 (98.1)	0.33*
Initial cure rate	*N* (%)	456 (99.1)	51 (96.2)	0.12
After initial cure is achieved				
Variables after initial cure	Parameters	VL *N* = 456	HIV–VL *N* = 51	*P* value
One or more relapses of VL within the first 6 months of follow-up	*N* (%)	30 (6.5)	18 (34)	< 0.001
Relapse-free survival at 12 months	*N* (%)	418 (90.9)	33 (64.7)	< 0.001
Mortality	*N* (%)	2 (0.4)	11 (21.6)	< 0.001

HIV–VL = human immunodeficiency virus–visceral leishmaniasis; SD = standard deviation.
*Comparison of proportions done with Fisher's exact test.

(95.5 ± 62 versus 100.7 ± 52.9, respectively, $P = 0.7$). However, at 6 months, patients on secondary prophylaxis had higher mean CD4 counts (196 ± 29.01 versus 157 ± 54.1, respectively, $P = 0.015$). Among patients not on secondary prophylaxis, those who did not experience relapses within first 6 months ($N = 6$) had higher mean CD4 counts than those who did in the first 6 months ($N = 18$) (201 ± 0.5 versus 143 ± 55.3, $P = 0.017$).

Primary and secondary outcomes. None in the secondary prophylaxis group versus 18/24 patients (75%, 95% CI = 55.1–88) without secondary prophylaxis had a relapse within the first 6 months of follow-up ($P < 0.001$). All patients with secondary prophylaxis (27/27) versus 6/24 (25%, 95% CI = 12–44.9) without secondary prophylaxis achieved 12-month relapse-free survival ($P < 0.001$). None in the secondary prophylaxis group versus 11/24 patients (45.8%, 95% CI = 27.9–64.9) without secondary prophylaxis died during follow-up ($P < 0.001$), the mean duration of survival before death being 10.4 ± 2.5 months (Figure 1).

Survival analysis. The profile of patients who died had the following characteristics significantly different from the survivors: lower hemoglobin level at baseline, lower mean CD4 cell count at 6 months of follow-up, lower proportion of patients receiving secondary prophylaxis, and higher proportion of patients experiencing at least one relapse within the first 6 months of follow-up (Tables 3 and 4).

Table 4 summarizes the results of the multivariate Cox regression for prediction of mortality in patients with HIV–VL. The following variables had P value < 0.2 in univariate analysis (Table 3) and were included in the Cox regression: hemoglobin level at baseline, total leukocyte count at baseline, CD4 cell count at 6-month follow-up, secondary prophylaxis, and relapse within the first 6 months of follow-up. Controlling for all other variables, secondary prophylaxis remained the sole significant predictor (HR = 0.09 [95% CI = 0.03–0.31] against death, $P < 0.001$) of survival.

DISCUSSION

This study highlights the importance of secondary prophylaxis in reducing relapses and mortality in patients with HIV–VL coinfection. This result is of vital importance as there are no well-designed studies in this area, especially from India, and our current understanding of HIV–VL coinfection is still in its infancy. Although HIV seropositivity among Indian patients with VL is around 1.5–6.3%[13–15]

TABLE 3
Comparison of demographics, clinical, and laboratory results of patients with HIV–VL coinfection who survived vs. those who died

Variables	Parameters	HIV–VL patients who died *N* = 11	HIV–VL patients who survived *N* = 40	*P* value
Age in years	Mean ± SD	35 ± 12	34 ± 7	0.67
Male gender	*N* (%)	9 (81.8)	31 (77.5)	0.56
Liver palpable below right costal margin (in cm)	Mean ± SD	4.3 ± 1.8	3.8 ± 1.9	0.41
Spleen palpable below left costal margin (in cm)	Mean ± SD	10.3 ± 3.6	9.3 ± 3.1	0.37
Hemoglobin at baseline (gm/dL)	Mean ± SD	6.5 ± 1.4	7.8 ± 1.6	0.01
Total leukocyte count (/μL)	Mean ± SD	2136 ± 724	2955 ± 1588	0.11
Platelet count at baseline (/μL)	Mean ± SD	1.2 ± 0.7	1.3 ± 0.6	0.71
CD4 cell count at baseline (/μL)	Mean ± SD	92.9 ± 62.6	103.4 ± 56.8	0.59
CD4 cell count at 6 months (/μL)	Mean ± SD	119.1 ± 40.1	191.2 ± 33.4	< 0.001
Patients who received secondary prophylaxis	*N* (%)	0	27 (67.5)	< 0.001
Tuberculosis coinfection	*N* (%)	0	7 (17.5)	0.32
One or more relapses of VL within the first 6 months of follow-up	*N* (%)	11 (100%)	7 (17.5)	< 0.001

HIV–VL = human immunodeficiency virus–visceral leishmaniasis; SD = standard deviation.

TABLE 4
Result of multivariable Cox regression with death as the dependent variable

Variables	B	SE	Wald	*P* value	HR	95% confidence interval for HR
Hemoglobin concentration at baseline	0.11	0.15	0.63	0.43	1.12	0.84–1.48
Total leukocyte count at baseline	0.00	0.00	0.07	0.78	1.00	1.0–1.0
Secondary prophylaxis received or not	–2.36	0.61	15.12	< 0.001	0.09	0.03–0.31
CD4 cell count at 6 months of follow-up	–0.003	0.01	0.24	0.63	1.00	0.98–1.01
Relapse within the first 6 months	0.65	0.66	0.97	0.32	1.91	0.52–6.95

B = Beta coefficient; HR = hazard ration; SE = standard error.

(5.6% from a recent cohort in Bihar, India),[16] clinical, diagnostic, and importantly, treatment outcome–related parameters are not well described in literature.

Most of our patients had presented with typical clinical features of VL. However, 28.3% had atypical clinical features, majority being gastrointestinal, pulmonary, and bleeding manifestation. Most important clinical features were high relapse rate (34% in first 6 months) and mortality rate (almost 25%). Similar results were also seen in a recent study by Burza and others from Bihar, India.[10] These essentially demonstrate that the natural history of VL with HIV as a backdrop is significantly different from VL monoinfection despite good compliance with ART.

Regarding diagnostics, an important ancillary result is utility of rK39 ICT in diagnosis of VL in people living with HIV/AIDS (positivity in serum 94.3%; positivity in urine 98.1%). We previously reported utility of urine rK39 test as one of the simplest noninvasive, field-adaptive, reliable test for diagnosis of VL with 100% sensitivity and 86.33% specificity.[17] In the setting of HIV–VL, Indian studies reported good efficacy of rK39 ICT for diagnosis of VL.[18] Our results indicate that rK39 works well in both serum and urine samples in Indian patients with VL with or without HIV. There was a statistically insignificant trend toward lower serum sample positivity in HIV–VL than in monoinfected patients with VL. It should be noted that rK39 ICT works better in Indian subcontinent compared with east African patients with VL.[19] The utility of urine rK39 as a minimally invasive technique might make it an attractive screening tool under field conditions.

The major challenges of HIV–VL are those of relapses and mortality. Almost one-third of our coinfected patients underwent at least one relapse within first 6 months and almost two-thirds within 1st year. Relapse rates ranging from 25% to 61%, depending on varying definitions used, have been reported previously.[10,20–24] A recent systematic review identified the following factors as predictors of relapse: failure of rise of CD4+ cells at follow-up, lack of secondary prophylaxis, and previous history of relapse of VL.[24]

Secondary prophylaxis has previously been tried in HIV–VL especially in Europe and Africa. The agents used for secondary prophylaxis were pentavalent antimony (24 patients),[25,26] liposomal AmB (14 patients),[26,27] AmB lipid complex (eight patients),[28] and pentamidine (75 patients, mostly from Ethiopia, uncontrolled).[26,29,30] No systematic study on secondary prophylaxis has been conducted in India, one of the largest contributors of the global burden of leishmaniasis. Despite uniformly low sample size and inconsistent designs in these studies, a high trend of relapse in coinfection is evident from these results. Regarding secondary prophylaxis antimonials, AmB and pentamidine appeared to render protection.[24–26,30] The doses used for liposomal AmB varied from 3 mg/kg/month (five patients),[27] with statistically insignificant protection against relapse to 200–350 mg monthly doses (nine patients),[26] with statistically significant protection against relapse. Considering average body weight of a typical VL patient in India being around 30 kg and 75th percentile being around 40 kg,[31] doses of 200–350 mg would amount to around 5–10 mg/kg/month, raising issues of toxicity and cost. Moreover VL caused by *Leishmania infantum* in Europe is traditionally treated with larger doses of liposomal AmB in comparison with *Leishmania donovani* in the Indian subcontinent. Considering toxicity, cost, and susceptibility, a dose of 1 mg/kg/month AmB for secondary prophylaxis has been in use in our institution (STM, Kolkata, India).

Initially, our patients with HIV–VL were managed conventionally (AmB + ART), but the problem of repeated relapses and mortality was evident to us within the first few years. In the present study, we therefore adopted the policy of secondary prophylaxis, on compassionate ground, which was subsequently adopted by few other physicians at the STM. HIV–VL coinfected patients were admitted to STM in different historical periods with similar clinical features. However, we could not find any other particular factor as indication for commencing secondary prophylaxis, apart from the intention to prevent relapses and mortality in the HIV–VL coinfected patients. Hence, majority of our patients who received secondary prophylaxis were from the later part of the retrospective study period.

The largest reported cohort of HIV–VL from India demonstrated low hemoglobin level and concurrent infection with tuberculosis as independent risk factors for mortality, with early ART reducing the risk of mortality and relapse in multivariate model.[10] Another recent report from Bihar, India, identified failure to initiate ART and concurrent tuberculosis as independent risk factors for mortality and poor outcome but could not identify any factors associated with relapse.[32] However, none of these groups offered secondary prophylaxis against VL. Tuberculosis occurred in 13.7% in our coinfected patients, but was not found to be a predictor of mortality. We demonstrated that secondary prophylaxis remained the singularly significant factor in reducing relapses and mortality after multivariate survival analysis (Cox regression).

Our study has certain limitations: low sample size, retrospective design, and heterogeneity of AmB preparations used for treatment, and possible bias toward more stringent follow-up in patients with HIV–VL coinfection.

In conclusion, this study demonstrates that initial treatment with AmB (usual total dose of 15–20 mg/kg) followed by ART and secondary prophylaxis with monthly AmB 1 mg/kg till CD4 rises above 200/μL can effectively prevent relapse and

decrease mortality in patients infected with HIV–VL in India. We believe that the results of this study will provide a major edge in the battle against HIV–VL coinfection, though further prospective randomized control trials are required in this area.

Received May 30, 2016. Accepted for publication October 19, 2016.

Published online November 22, 2016.

Acknowledgments: We are grateful to Indrajit Chatterjee, Consultant Physician in Geriatric and General (Internal) Medicine, Glan Clwyd Hospital, Wales, United Kingdom, for kindly consenting to review the manuscript for English language usage, context, and overall editing. The American Society of Tropical Medicine and Hygiene (ASTMH) assisted with publication expenses.

Disclaimer: Part of the results of this study were presented at MSF Scientific Day, New Delhi, India, 2015.

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