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Research Article

EPIDEMIOLOGICAL STUDIES ON LEISHMANIASIS IN MURSHIDABAD DISTRICT OF WEST BENGAL, INDIA

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ABSTRACT

The study was conducted to find out the prevalence of VL, PKDL and asymptomatic VL and to detect sero-conversion rate of asymptomatic VL case and the role of liposomal amphotericin-B in the treatment of VL and PKDL cases which in-turn help to reach our national goal of VL elimination. Population of study villages were examined by rK39 kit for detection of VL, PKDL and asymptomatic VL cases. Detected asymptomatic VL infections were followed-up for 1.5years to observe sero-conversion pattern. Among 2224 individuals 127 were rK39 positive, of which 72 had previous history of Kala-azar and 55 without Kala-azar history. Among 55 rK39 positive cases-VL, asymptomatic VL and PKDL was confirmed in 1.81%, 96.36% and 1.81% cases, respectively. Both VL and PKDL cases were successfully treated with amphotericin-B. Among 53 asymptomatic VL, 73.58% became negative spontaneously during 1.5 years and 7.54% still remained positive. During study period 17 new infections were as detected. Active mass screening helps to detect VL, PKDL and asymptomatic cases. Ongoing transmission of the disease as evidenced by newly infected individuals perhaps due to presence of asymptomatic cases. So, asymptomatic VL and PKDL cases need much attention to reach our goal of Kala-azar elimination.

INTRODUCTION

Visceral leishmaniasis (VL), or Kala-azar, is a vector-borne parasitic disease (Ahluwalia *et al.,* 2003) and one of the world's most neglected and poverty-related diseases, affecting the poorest people in tropical and subtropical countries (WHO, 2017). In India, it is caused by the flagellated hemoparasite *Leishmania donovani* and is transmitted by the bite of the infected female Phlebotomine sandfly (Sanyal RK. 1985). Kala-azar is spread over a large geographical area across the globe with estimated yearly incidence of 500,000 cases, which lead to loss of nearly 2.4 million disability-adjusted life years (DALYs) each year (WHO, 2015).

In the WHO South-East Asian Region Kala-azar is prevalent in India, Bangladesh and Nepal and with few foci in Bhutan (WHOSEAR, 2015). In India it is endemic in 52 districts spread over four states namely Bihar, Uttar Pradesh, West Bengal and Jharkhand with over 165.4 million people at risk (NVBDCP, 2017). Particularly in West Bengal 11 districts are endemic for the disease. Regardless of lots of strategy for elimination estimated annual incidence of Kala-azar cases are varying from 20 - 25 per 10,000 population in India (Singh *et al.,* 2011). Kala-azar had re-emerged in the Indian

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subcontinent from near eradication phase which was achieved due to intensive vector control measures adapted under the Malaria Eradication Program (MEP) during 1960s (Bora, 1999). In India there is a resurgence of Kala-azar in last few years, but overall deaths are declining (NVBDCP, 2017; WHOSEAR, 2017). Due to increasing incidence of Kala-azar, the Government of India, launched a centrally sponsored "Kala-azar Control Program" during 1991 (Kishore *et al.,* 2006). In the year 2005, the Kala-azar Control Program was merged with "National Rural Health Mission" under the name of National Vector Borne Diseases Control Program (NVBDCP) (Singh *et al.,* 2006) and set a goal for the "elimination of Kala-azar by the end of the year 2015" under Millennium Development Goals (MDG).

The Kala-azar elimination programme is aimed to reducing the incidence of Kala-azar to < 1/10,000 populations at the district level (Gupta *et al.,* 2015). The main strategies of Kala-azar elimination programme are early diagnosis and complete case management, integrated vector management (IVM) with the focus on indoor residual spraying (IRS), effective disease surveillance through passive and active case detection and vector surveillance, social mobilization and building partnerships and operational research (WHOSEAR, 2017). But the programme is facing lots of challenges to achieve the goal such as long incubation period of the parasite, ignorance of the disease by patients, co-infections like HIV, lack of proper and reliable diagnostic methods particularly for asymptomatic VL and PKDL cases.

Symptomatic cases of VL and cases of post Kala-azar dermal leishmaniasis are considered potential reservoirs of VL, and thus play a major role in transmission of the disease in VL-endemic areas. Most of the infected population in whom VL does not develop is considered asymptomatic; however, these cases can act as potential reservoirs in transmission of VL (Sharma *et al.,* 2000). However, the actual estimation of asymptomatic cases in a VL-endemic area is difficult to assess. As the asymptomatic VL and PKDL cases do not seek any health care facilities, so need to address these hidden parasite pool to reach the goal of elimination of Kala-azar. The present study was conducted to detect the prevalence of VL, PKDL and asymptomatic VL in an endemic area of Murshidabad district, West Bengal, India by active mass survey.

MATERIALS AND METHODS

Study sites: The study was conducted in Samserganj and Sagardighi blocks of the district of Murshidabad. Two villages of Samserganj block (Dogachhi and Jethkunda) and four villages of Sagardighi block (Khatua Bhutindanga, Beldanga Adibasipara, Tikhordanga, Hukarhut Paschimpara) of Murshidabad district were selected as study area based on information from local health authorities (Figure 1). Active surveillance was done for leishmaniasis by the detection of anti-leishmanial antibody using rK39 based rapid diagnostic kit.

Treatment and Follow up: During the study period, both VL & PKDL patients were treated with liposomal amphotericin-B as per NVBDCP guidelines. A single dose infusion was administered at 10mg/kg of body weight in VL and at 5 mg/kg of liposomal Amphotericin-B infused intravenously, twice weekly, for 3 consecutive weeks for a total dose of 30 mg/kg in subjects with PKDL. To ascertain the adherence, all treatment was supervised by local trained volunteers.

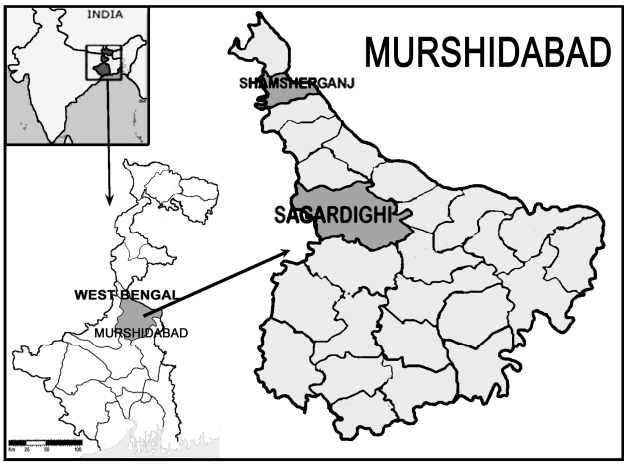

Figure 1 Map showing the study area and study sites

Laboratory Methods

Rapid Diagnostic Test: During surveillance, the study population was screened for anti-leishmanial antibody by rk39 strip test by trained research project staff using finger prick blood as per the manufacturer's instructions.

Collection of slit skin scraping for microscopy: Slit skin scraping samples were collected from the suspected PKDL patients following necessary aseptic measures. The materials were thinly spread on three clean glass slides using a circular motion working outwards to avoid damaging any parasites. When the smears dried, two slides were fixed with a few drops of absolute methanol for 2-3 minutes. The remaining slide was heat-fixed and stained with modified Ziehl-Neelsen (Z-N) staining (using 5% sulphuric acid, H_2SO_4). It was then examined under oil immersion lens using 100x oil immersion objectives to look for *Mycobacterium leprae*. The methanol-fixed slides were stained with Giemsa and examined for LD bodies.

Case definition and detection

VL: A person from an endemic area with fever of more than two weeks duration and with splenomegaly, who is confirmed by an RDT or a bone marrow or spleen biopsy.

Asymptomatic VL: A person from an endemic area with no signs and symptoms of VL (such as fever of more than two weeks duration and with splenomegaly), but who is antibody positive by RDT (Rapid diagnostic test).

Probable PKDL: A patient from a KA-endemic area with multiple hypopigmented macules, papules, plaques or nodules and is RDT positive.

Confirmed PKDL: A patient from a KA-endemic area with multiple hypopigmented macules, papules, plaques or nodules, is parasite positive in slit-skin smear.

RESULTS

Demography of the study population: In all villages under study area, the houses were made of either mud or brick. The source of drinking water was tube-well and household works were done mostly by pond water. Most of the houses have cattle sheds. Pig rearing was the common practice in the study villages of Sagardighi block. Majority of the people were

agricultural or migrant labourers. The demographical data of the study population is listed in Table 1.

Table 1 Demographical information of the study villages

Districts	Blocks	Village Name	No. of Households	Population	Gender distribution	
					Male	Female
Murshidabad	Sagardighi	Khatua Bhutindanga	94	386	190	196
		Beldanga Adibasipara	112	537	260	277
		Tikhordanga	109	491	242	249
		Hukarhut Paschimpara	39	207	102	105
	Shamserganj	Dogachhi	46	346	166	180
		Jethkunda	120	523	248	275

Outcomes of 1st Mass Survey: During the 1st Mass survey, carried out during March - April 2014, a total of 2224 among 2491 individuals were screened by RDK (rK-39) of which 127 individuals were positive for anti-leishmanial antibody. Among those 127 RDK positive subjects, 72 had previous history of Kala-azar (KA) while rest 55 did not have any past history of KA. Of those 55 subjects, VL & PKDL was confirmed in 1 case each. The remaining 53 cases were diagnosed as asymptomatic VL and followed-up. Among the 72 cases having previous history of KA, 2 cases were diagnosed as PKDL. All diagnosed VL & PKDL cases were suggested to treat accordingly by the local heath authority (Figure 2).

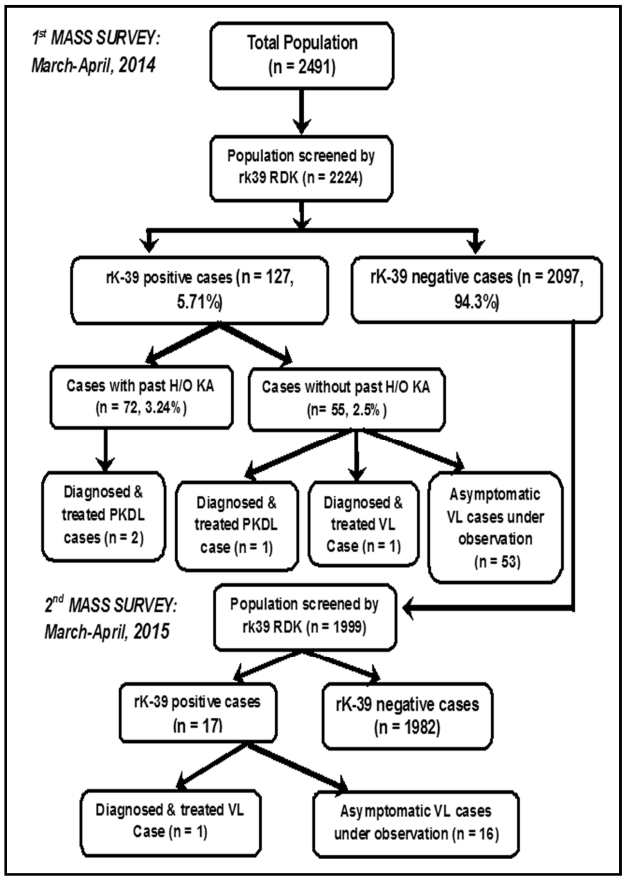

Figure 2 Flow Chart showing the Summary of 1st and 2nd mass Survey

Outcomes of 2nd Mass Survey: During the 2nd Mass survey, among the 2097 initially anti-leishmanial antibody negative subjects, a total of 1999 were screened by RDK (rK-39) of which 17 were detected to be positive for anti-leishmanial antibody. Among those 17 subjects, VL was diagnosed in one. The rest 16 were diagnosed as asymptomatic VL and followed-up. No new PKDL cases were diagnosed during the 2nd mass survey (Figure 2).

Follow up of asymptomatic rK-39 positive cases of 1st Mass Survey: During the 1st active Mass Survey, there were 55 rK-39 positive cases without having any past history of Kala-azar. Among them, one was diagnosed as active VL and one as PKDL. Remaining 53 individuals were classified as Asymptomatic VL and followed up. After 7 months of follow up, no new VL was detected among the 53 asymptomatic VL cases. Five (5) of them became negative for Anti-leishmanial antibody. Remaining 48 were continued to be followed up. Following 1 year of follow up, none of the 48 asymptomatic VL subjects was found to have active VL. Sixteen of them became negative for Anti-leishmanial antibody. Remaining 32 cases were further followed up. At the end of 1.5 years of follow-up, no new VL cases developed among the 32 asymptomatic VL subjects. While 18 of them became negative, 4 continued to be positive for anti-leishmanial antibody. Ten (10) migrant workers were lost to follow-up (Figure 3).

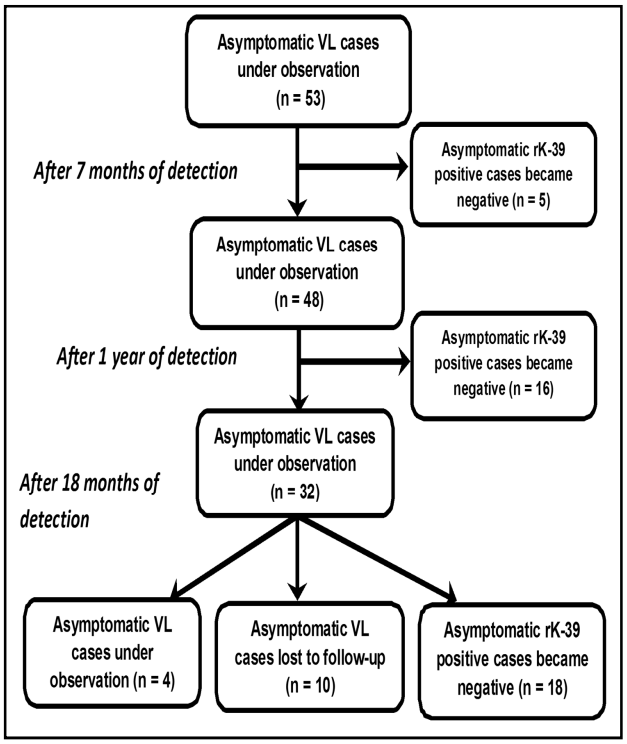

Figure 3 Summary Chart of Follow-up of 1st Mass Survey Asymptomatic VL Cases

DISCUSSION

In India attempts were made thrice to eliminate VL but failed due to several reasons (Bora, 1999; Thakur *et al.,* 2009). The first attempt was made through National Malaria Eradication Program (NMEP) which initiated an intensive use of DDT spraying during 1953 and 1964 for malaria eradication programme. The number of VL cases declined from 60,000 to almost zero during 1955-1956 (Thakur, 2007). Second attempt was done through "Kala-azar control program" launched in 1977. DDT spraying was done for 3 years between 1977 and 1979 and discontinued at the end of 1979 (Thakur, 2007). Third attempt was taken during 1991-92 outbreaks by centrally sponsored "Kala-azar control program". The DDT spraying was started in 1992 and again discontinued in 1995 (Thakur, 2007). In all three attempts DDT played the central role. Then it was hypothesised that in India VL epidemic would occur as a wave, once in every 10-15 years (Alvar *et al.,* 2006) and the reason behind this was not well explained. Perhaps parasites persist in the community in the form of PKDL and asymptomatic infection. In favourable ecological condition this hidden parasite pool is responsible for new epidemics (Addy and Nany, 1992; Desjeux *et al.,* 2013).

The elimination of VL from Indian subcontinent is feasible due to the following reasons: the disease is anthroponotic in nature, and *P. argentipes* the only known vector, new tools for diagnosis i.e. rK39 dipstick and availability of an effective oral drug miltefosine for case management (WHO, 2005). Beside these VL is restricted in 56 districts in India, 12 districts in southeastern Nepal, and 31 districts in Bangladesh (Huda *et al.,* 2011). Due to unavailability of an effective anti-leishmanial vaccine, early diagnosis and case management strategies including integrated vector control are the main strategies of the ongoing elimination programme.

To achieve early diagnosis and complete case management, rK39 dipstick test and miltefosine are being made freely available at public health facilities in the endemic areas. Amphotericin B and lipid formulations of amphotericin B (Ambisome) are kept as 2nd line treatment; paromomycin as possible alternative (WHO, 2005). Integrated vector control (IRS and ITNs) is one of the pillars of the current VL elimination programme, alongside early case detection and treatment. The aim of vector control programme is to reduce or interrupt transmission of disease. An effective strategy for reducing VL incidence is to control sand fly vectors, especially in domestic and peridomestic transmission habitats. A number of control methods are available, including chemicals, environmental management and personal protection (Ganguly *et al.,* 2015). VL control has often been integrated with that of other vector borne disease. For example, after intensive attempts to eradicate malaria in the 1950s and 1960s by IRS with DDT, the prevalence of VL fell dramatically in many countries. In this approach, integrated vector management programmes combine interventions and resources and target several vector-borne diseases (e.g. malaria, dengue, filariasis) in one area. However, there are number of reports about DDT resistance in sandflies (Kishore *et al.,* 2006).

In Murshidabad district, all interventions were implemented as per NVBDCP guidelines. In spite of that new VL cases were diagnosed with a reduced incidence rate. During the present study we diagnosed three VL and three PKDL cases by door to door mass survey. PKDL cases were apparently normal except dermal lesions; they do not seek any medical treatment initially. These cases are suspected as the source of transmission as parasites are easily available to the vector sand fly from the skin lesions (Addy and Nandy, 1992; Desjeux *et al.,* 2013; Ganguly *et al.,* 2015). Apart from this asymptomatic cases might be the source of disease transmission. During our study period we observed that four asymptomatic VL cases remained positive for antileishmanial antibody after 18 months of their initial diagnosis. Saha *et. al.*, 2017 (Saha *et al.,* 2017) reported 10.4% disease conversion among such asymptomatic cases during three years of follow up from Malda district, adjoining to the present study areas. It indicates that asymptomatic VL cases harbours parasite and acts as a source of disease transmission. Several workers detected parasitic DNA by PCR from blood samples of such asymptomatic cases with a varying rate (Abbasi *et al.,* 2013; Silva *et al.,* 2013; Sudarshan *et al.,* 2014; Srivastava *et al.,* 2013). In the present study we did not attempt to detect parasitic DNA, so it is not possible to mention about persistence of parasite among the studied asymptomatic cases. Most important observation was the diagnosis of 17 new asymptomatic cases by detecting antileishmanial antibody among the individuals those was negative during first mass survey. One such case was also diagnosed as VL by the demonstration of parasite in bone marrow sample. It indicates that disease transmission was going on during the study period. Previously a high rate of new infection in an interval of one year was recorded from South 24-Parganas district of the same state (Saha *et al.*, 2009). Perhaps asymptomatic VL and PKDL cases act as the source of parasite for disease transmission. So, importance should be given to such asymptomatic VL and PKDL cases to reach the goal of VL elimination from this part of the World.

Author Contribution

SKG and AKM designed the study; SBD, PS, MC, SG, AC and NG performed door to door survey, clinically examined, recruited and followed up the patients, collected the blood samples; SKG, SG, AC, NG provided the treatment, SBD, PS, MC, SG, assessed laboratory diagnostics. MC, PS, SBD perform the data analysis and interpretation. SKG, AKM, SG prepared the manuscript.

Conflict of Interest

We have no conflicts of interest concerning the work reported in this article.

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