VISCERAL LEISHMANIASIS IN A NON-ENDEMIC REGION OF INDIA- INVESTIGATION OF AN OUTBREAK

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ABSTRACT**BACKGROUND**

Visceral Leishmaniasis (VL) is seen commonly in eastern parts of India, while occurrences of cutaneous forms of the disease have been reported recently from Western Ghats of southern India. The clinical manifestations of VL resembles many other chronic illnesses and its diagnosis and management requires added attention in southern parts of the country. The clinical, epidemiological and entomological investigations related to the occurrence of a case of VL in Kerala, south India are described in detail.

MATERIALS AND METHODS

This study is a descriptive study. The patient suffered from various clinical manifestations which were investigated repeatedly at various primary, secondary and tertiary care institutions. Several laboratory tests for multiple diseases turned out to be negative, even though the symptoms were persisting. Accurate diagnosis was made from the bone marrow cytological examination, which indicated the presence of Leishman-Donovan bodies. Further epidemiological and entomological investigations confirmed the presence of vector (*Phlebotomus argentipes*) from multiple locations around the residence of the patient. Evidence of Leishmaniasis or its source could not be diagnosed in contacts, vectors or suspected animal reservoirs in the area.

RESULTS

Accurate diagnosis was made from the bone marrow cytological examination, which indicated the presence of Leishman-Donovan bodies. Epidemiological and entomological investigations confirmed the presence of vector (*Phlebotomus argentipes*) from multiple locations around the residence of the patient. Evidence of Leishmaniasis or its source could not be diagnosed in contacts, vectors or suspected animal reservoirs in the area.

CONCLUSION

Early and accurate diagnosis of VL is difficult in south India due to unawareness about the manifestations of disease and also due to unavailability of reliable laboratory kits. The management of such cases is even more challenging due to the lack of availability of proper drugs. The recent reports of emergence of resistant cases should be a pointer in creating improved awareness and facilities for the rapid identification and management of cases of visceral leishmaniasis.

KEYWORDS

Visceral Leishmaniasis, Leishmaniasis in Kerala, Kala-Azar in Kerala, Epidemiological Investigation of Leishmaniasis.

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BACKGROUND

Leishmaniasis is a slowly progressing parasitic disease caused by a protozoan parasite of genus *Leishmania*. It is transmitted by the bite of an infected female *Phlebotomus* (sand fly) and is seen mostly in developing countries.^{1,2} It can manifest as cutaneous, mucosal or visceral forms, which result from infection of macrophages in the dermis, in the naso-oro-pharyngeal mucosa and throughout the reticuloendothelial system, respectively. Cutaneous and mucosal leishmaniasis causes substantial morbidity, whereas visceral leishmaniasis (VL) can be life threatening.³

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In India visceral leishmaniasis or kala-azar is prevalent in the eastern states having hot and humid climate.⁴ Abundance of this disease is normally recorded during the period from June to September in India.⁵ Isolated occurrence of VL has also been reported from Maharashtra in migrant labourers from south-east Asia.¹ Cutaneous forms of the disease are now being detected increasingly in the southern part of the country.² Newer species of *Phlebotomus* and natural infection of *Phlebotomus* with *Leishmania* parasites have been demonstrated in the tribal belts of western ghats in south India in recent years.^{6,7} Cutaneous and visceral forms of the disease have been detected in individuals with no history of travel to endemic areas and in whom no definite routes of transmission could be postulated.^{2,7} Also, the clinical management of VL is undergoing rapid changes with the use of liposomal forms of Amphotericin B and also due to the use of various combinations of miltefosine, amphotericin B, paromomycin etc.⁸ But the healthcare professionals are facing newer challenges due to the emergence of miltefosine resistant forms of VL.⁹ This points to the need for early identification and management of suspicious cases by

healthcare professionals. Following the serendipitous discovery of visceral leishmaniasis at a tertiary care hospital the study aimed at epidemiological and entomological investigations for cases, carriers and infected vectors in a human-forest interface in Kollam district of Kerala in India. The objective of the study was the epidemic investigation of the case of visceral leishmaniasis identified at a tertiary health centre in Kerala, India.

MATERIALS AND METHODS

The study was descriptive in nature conducted at a rural area of Kollam district, Kerala, India.

Clinical Investigation

The patient was a 63-year-old housewife residing in 'Kadambupara' near 'Chempanaruvu' of Kollam District of Kerala. Chempanaruvu is a small village located in the foothills of Western Ghats, geographically linked to places recently reported to have heavy infestations of sandflies.^{6,7} Clinical examination and laboratory investigations and bone marrow biopsy were carried out for confirmation of diagnosis. Polymerase Chain Reaction (PCR) analysis was carried out at the Vector Control Research Centre (VCRC) at Kottayam, Kerala to characterise the parasite species involved in the infection. A multiple genetic marker approach (mini-circle kinetoplast DNA, restriction analysis of 3'UTR of HSP70 and HSP70 gene sequence analysis) was adopted¹⁰ for the same. The DNA sequences of the HSP70 gene of the isolate was submitted with the GenBank.

Epidemiological Investigation

Epidemiological survey of patient's house and surroundings were initiated at the time of histopathological diagnosis to investigate the case and to confirm the presence of sand fly, which is the vector of the disease. The locality is inside a forest area with thick plantations and most of the houses are having non-plastered walls. Most of the people had occupations related to adjacent forest area. A thorough search of the area was conducted during daytime around the patient's residence. These included adjacent houses, cattle sheds, uninhabited constructions etc. A screening camp was conducted 10 days later to obtain further evidence regarding any undetected cases in the area. Extensive Information Education and Communication (IEC) activities by the local self-government as well as the district health authorities resulted in large participation of the local people from varying social and geographic affinities. Patients with various clinical complaints like fever, rashes, skin pigmentation, fatigue etc. were examined by a Physician and a Dermatologist. Any person with a prolonged history of fever, suspicious skin pigmentation etc. was examined in detail by the team from the Department of Community Medicine, Government Medical College Hospital, Trivandrum. Data from suspected cases were collected by the investigating team using a structured questionnaire. The case definition used to screen for visceral leishmaniasis (Kala-Azar) included fever for more than 2 weeks duration, pallor, palpable spleen, papular/ nodular cutaneous lesions.

Simultaneously, four contacts (2 males and 2 females) who were residing in the houses adjacent to the house of the index case were identified. The contacts were visited at their homes by the team and blood samples were drawn after

obtaining informed written consent. One sample from each contact was taken for PCR, blood count and peripheral smear examination. The animal husbandry department collected 4 blood samples from animals (2 cattle and 2 goats) in the locality of the house of the index case, which was also analysed using PCR. Blood samples from dogs could not be obtained.

RESULTS

Clinical Investigation

The patient initially presented with history of persistent low-grade fever and fatigue for past months during September 2015. Despite seeking treatment at various government and private hospitals, her symptoms did not subside. She had comorbidities like dyslipidaemia and type 2 diabetes mellitus since 2008 and was on regular medications. In March 2016, laboratory investigations done at Government Medical College Hospital, Kottayam, Kerala did not reveal any conclusive diagnosis. Bone marrow study showed Myeloid: Erythroid= 2.5: 1, mainly marrow blood, erythroid-normoblastic maturation, myeloid mature forms reduced with collection of immature cells. Occasional megakaryocytes with normal morphology was seen. Abdominal ultrasonography showed spleen to be 17.5 cm and splenic vein to be 9 mm in size and a normal sized liver (10 cm) with normal echotexture. The details of initial investigations done are given in Table 1. She was subsequently sent home.

As the fever did not subside and fatigue was increasing, the patient was referred from the local hospital to Government Medical College Hospital at Trivandrum, Kerala by April 2016. At the time of admission, she had severe pallor and splenomegaly. Chest Radiology and ECG were found to be within normal limits. Peripheral smear showed anisopoikilocytosis with normocytes, macrocytes, microcytes, ovalocytes, occasional spherocytes; normochromic and mildly hypochromic RBCs, increase in Rouleaux formation; severe leucopenia; neutrophils and lymphocytes almost equal with occasional monocytes; platelet count reduced and reported as pancytopenia. Ultrasonographic imaging of abdomen showed massive splenomegaly (18.5 cm x 9 cm) with no ascites or any other significant findings. Anti-nuclear antibody, DCT, RMT were also not significant. Hepatitis B, Hepatitis C, HIV, typhoid fever, scrub typhus, leptospirosis and dengue fever were ruled out by appropriate laboratory investigations. Blood culture and urine Bence Jones proteins were also negative. Serum electrophoresis showed increased gamma globulin fraction and reduced albumin. Vitamin B12 and folate levels were 545 pcg/mL (normal range: 187 - 883 pcg/mL) and 6.7 ng/mL (normal range: 3.1 - 20.5 ng/mL) respectively. The significantly low blood count along with the above negative results led to a provisional diagnosis of multiple myeloma. Bone marrow aspiration cytology (trephine biopsy) was done for confirmation, which revealed Leishman Donovan (LD) bodies in macrophages, diagnostic Visceral Leishmaniasis (Figure 1 with detailed report). Polymerase Chain Reaction (PCR) analysis carried out evinced that the parasite species involved in the infection to be *Leishmania donovani*. No findings suggestive of multiple myeloma were visualised in the bone marrow biopsy.

Treatment was started with Amphotericin B infusion at 25 mg in 5 percent Dextrose for 3 days. Subsequent doses of 30 mg for 1 day, 40 mg for 1 day and 50 mg for 6 days were

given. Blood Routine Examination and Renal Function Tests were being monitored (Table 1), which showed that the patient developed non-oliguric Acute Kidney Injury (AKI). The treatment was changed to Liposomal form of Amphotericin B at 150 mg for 5 days and Miltefosine 50 mg tablet two times daily for 28 days. Potassium and Magnesium supplementation was also initiated promptly. The patient responded favourably to treatment and was discharged.

Epidemiological Investigation

Phlebotomus argentipes, the established vector species was not detected inside the house of the index case or in the nearby houses/ cattle sheds. But they were detected from several houses in a tribal colony situated within 2 km from the patient's residence (Figure 2 and Figure 3). Out of the 56 houses surveyed, 52 (92.9%) had at least one un-plastered inner wall and a similar number (n= 53, 94.6%) had crevices inside houses. Dogs were present in two-thirds of the household, while 20% of the households had domestic animals inside the houses. Twenty six houses (46.4%) were

found to have sand-flies within them. Details of the household survey are given in Table 2. Individuals with lesions suggestive of past infections of cutaneous forms of the disease could also be seen during this phase of the investigation.

A total of 346 individuals attended the screening camp. Rapid diagnostic kit test (rK39) was applied to 7 persons who fulfilled the case definition including the daughter and grandson of the index case, but all were found to be negative. Blood samples were collected from 6 more additional suspects for a detailed blood count and peripheral smear evaluation. The blood counts and the peripheral smear of none of the 13 suspects showed any evidence for the presence of *Leishmania*.

PCR analysis of sand-flies collected from various households in the locality and the blood samples of the human contacts also failed to show any evidence for the presence of *Leishmania*. The 4 blood samples collected from suspected animal reservoirs also failed to show any confirmatory evidence for *Leishmania* infection.

Laboratory Parameter	15 th March, 2016	16 th April, 2016	18 th April, 2016 (Admission)	27 th April, 2016	2 nd May, 2016	10 th May, 2016	24 th May, 2016 (Discharge)
Haemoglobin	9 gm/dL	7.5 gm/dL	8 gm/dL	8.3 gm/dL	9.5 gm/dL	9 gm/dL	11.4 gm/dL
Total Count	4100/dL	2300/dL	3210/dL	2900/dL	5600/dL	5200/dL	4700/dL
Platelet Count	51000/dL	45000/dL	40000/dL	55000/dL	69000/dL	88000/dL	136000/dL
ESR	90 mm/hr	98 mm/hr	117 mm/hr	57 mm/hr	46 mm/hr	40 mm/hr	50 mm/hr
Blood Urea	30 mg/dL	36 mg/dL	29 mg/dL	34 mg/dL	48 mg/dL	32 mg/dL	28 mg/dL
Serum Creatinine	1 mg/dL	1 mg/dL	0.9 mg/dL	1.4 mg/dL	2.6 mg/dL	1.2 mg/dL	0.9 mg/dL
Serum Potassium	3.8 mmol/L	3.6 mmol/L	3.9 mmol/L	3.6 mmol/L	3 mmol/L	3.4 mmol/L	3.8 mmol/L
Serum Magnesium	-	-	1.36 mg/dL	1.2 mg/dL	1.4 mg/dL	1.5 mg/dL	1.9 mg/dL

Table 1. Progress of Laboratory Parameters

Sl. No.	Details	Frequency (%)
1	Total Houses surveyed	56 (100)
2	Kutch houses	49 (87.5)
3	Houses with at least one inner unplastered wall	52 (92.9)
4	Houses with crevices inside houses	53 (94.6)
5	Presence of cattle sheds	20 (35.7)
6	Presence of cattle	16 (28.6%)
7	Presence of domestic animals inside houses	11 (19.6%)
8	Presence of dogs in the household	37 (66.1%)
9	Mean (Range) number of dogs	1.8 (1-6%)
10	Houses with presence of sand-flies	26 (46.4%)

Table 2. Details of Initial Household Survey

Symptoms	Affected Individuals (n= 346)
Fever	41 (11.85%)
Rash	3 (0.9%)
Skin pigmentation	5 (1.4%)
Fatigue	3 (0.9%)
Pallor	22 (6.3%)
Jaundice	0
Lymph node enlargement	3 (0.9%)
Hepatomegaly	3 (0.9%)
Splenomegaly	1 (0.3%)
Presence of nodule	0
Pain in nodule	0
Itching in nodule	0
Ulceration of nodule	0

Clustering of nodule	0
Oozing from the nodule	0
Sensory loss over the nodule	0
Any treatment taken for the nodule	0
Clustering of nodule	0
Oozing from the nodule	0
Table 3. Screening of Subjects at Medical Camp

Figure 1. Leishman Donovan Bodies in Bone Marrow Cytology of Patient

- M: E= 1.5: 1. Erythroid in normoblastic and megaloblastic maturation.
- Myeloid in all stages of maturation.
- Megakaryocytes proliferated, seen in normolobated forms.
- Plasma cells (6%) seen, mature forms, distributed singly, bare nuclei seen.
- Histiocytes seen with inclusions resembling LD bodies. Extracellular structures also seen.
- BM iron - 5+ (increased).
- Impression: Erythroid, myeloid, megakaryocytes in normal morphology. Plasmacytosis noted. Also seen are many histiocytes packed with LD bodies. Impression of Visceral Leishmaniasis.

Figure 2. Phlebotomus argentipes obtained during Household Survey

Figure 3. Geographic Locations where presence of Phlebotomus was Seen

DISCUSSION

Leishmaniasis could be considered as one of the emerging diseases in southern part of the country, as two cases of visceral leishmaniasis have been confirmed in two patients from the district of Malappuram in Kerala in the recent past.⁷ The patient had a presentation with anaemia, hepatosplenomegaly and other non-specific symptoms which could only be diagnosed after long and tedious laboratory and histopathological investigations. The repeat bone marrow sampling and Polymerase Chain Reaction (PCR) analysis of the blood sample of the patient proved the presence of parasite. The initiation of treatment with amphotericin B initially improved the clinical condition. But symptoms and signs suggestive of acute kidney injury prompted administration of liposomal form of AMB and later with miltefosine tablets. Various combinations containing both amphotericin and miltefosine are widely used in the management of visceral leishmaniasis.⁸ But the recent evidences of lab-confirmed miltefosine resistant visceral leishmaniasis should be a pointer in the adoption of comprehensive treatment strategies for the disease.⁹

The longer duration of illness in this patient and in other non-endemic areas are mostly due to the delay in diagnosis. The similarity of presenting symptoms to other zoonosis and the unawareness about the epidemiology of the disease in the present clinical setting could have contributed to this delay in diagnosis. It lowers immunity, causes persistent fever, anaemia, liver and spleen enlargement, and if left untreated can result in death due to opportunistic infections. The gradual improvement in symptoms and blood parameters with treatment shows the need for early initiation of therapy for prevention of morbidity and mortality. Tracing of contacts and their laboratory evaluation including PCR, failed to find any cases or carriers for the disease. The presence of multiple risk factors could have caused the exposure of this patient to infected Phlebotomus bite. The unavailability of rapid diagnostic kits/ facilities is a hindrance in rapid identification and diagnosis of suspicious cases.

Phlebotomus argentipes, the most important vector for Visceral Leishmaniasis could be found in large numbers from several houses around the residence of the patient. The area is near to forest land with thick vegetation and people are having close contact with the vegetation. Most of the people are in close contact with forest for their daily occupation, which has been identified as a major factor determining infection and mortality.⁴

The topography of the locality along with the nature of human habitats are important determinants for the presence and survival of *Phlebotomus*. In this locality, inner side of the walls of the houses are not plastered resulting in several cracks and crevices within the house itself. This is conducive to the breeding of sand fly as has been shown previously.¹¹ Animal reservoirs have been postulated to be an important factor in the epidemiological triad associated with leishmaniasis. This has been proven in similar setting in Kerala in the recent past with respect to dogs.¹² In the present investigation, presence of animal reservoirs could not be established despite efforts to establish the same. The inability to obtain blood samples from dogs despite several attempts has limited the study for animal reservoirs.

Indoor residual spraying has been found to be a very effective short-term measure during the course of the epidemiological investigation. But the coverage of the spraying activities does not seem to be sufficient for effective vector control as sandflies were detected from houses in adjoining areas. Attempts for elimination of visceral leishmaniasis from the south-east Asian region could only be attained if intersectoral collaboration occurs among professionals in health, animal husbandry and entomology for rapid identification of cases and vectors.¹³ The abundance of vectors in this geographical area points to the need for effective vector control measures as well as awareness of generation about leishmaniasis.

CONCLUSION

Early and accurate diagnosis of VL is difficult in south India due to the unawareness about the manifestations of disease and also due to the unavailability of reliable laboratory kits. The management of such cases is even more challenging due to the lack of availability of proper drugs. The emergence of new cases along with recent reports of emergence of resistant cases in other parts of India should be a pointer in creating improved awareness and facilities for the rapid identification and management of cases of visceral leishmaniasis.

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REFERENCES

- [1] Gawade S, Nanaware M, Gokhale R, et al. Visceral leishmaniasis: a case report. *Australas Med J* 2012;5(2):130-4.
- [2] Simi SM, Anish TR, Jyothi R, et al. Searching for cutaneous leishmaniasis in tribals from kerala, India. *J Glob Infect Dis* 2010;2(2):95-100.
- [3] Prevention C-C for DC and CDC - Leishmaniasis - resources for health professionals. 2017. http://www.cdc.gov/parasites/leishmaniasis/health_professionals/index.html
- [4] Huda MM, Chowdhury R, Ghosh D, et al. Visceral leishmaniasis-associated mortality in Bangladesh: a retrospective cross-sectional study. *BMJ Open* 2014;4(7):e005408.
- [5] Dhiman RC. Emerging vector-borne zoonoses: eco-epidemiology and public health implications in India. *Front Public Health* 2014;2:168.
- [6] Ranganathan S, Swaminathan S. Sandfly species diversity in association with human activities in the Kani tribe settlements of the Western Ghats, Thiruvananthapuram, Kerala, India. *Mem Inst Oswaldo Cruz* 2015;110(2):174-80.
- [7] Visceral leishmaniasis in Kerala: an emerging Disease 2017. http://njcmindia.org/uploads/7-8_722-724.pdf
- [8] Rahman R, Goyal V, Haque R, et al. Safety and efficacy of short course combination regimens with AmBisome, miltefosine and paromomycin for the treatment of visceral leishmaniasis (VL) in Bangladesh. *PLoS Negl Trop Dis* 2017;11(5):e0005635.
- [9] Srivastava S, Mishra J, Gupta AK, et al. Laboratory confirmed miltefosine resistant cases of visceral leishmaniasis from India. *Parasit Vectors* 2017;10(1):49.
- [10] Kumar NP, Srinivasan R, Anish TS, et al. Cutaneous leishmaniasis caused by *leishmania donovani* in the tribal population of the Agasthyamala Biosphere Reserve forest, Western Ghats. Kerala, India. *Journal of Medical Microbiology* 2015;64(Pt 2):157-63.
- [11] Perry D, Dixon K, Garlapati R, et al. Visceral leishmaniasis prevalence and associated risk factors in the saran district of Bihar, India, from 2009 to July of 2011. *Am J Trop Med Hyg* 2013;88(4):778-84.
- [12] Jambulingam P, Kumar PN, Nandakumar S, et al. Domestic dogs as reservoir hosts for *Leishmania donovani* in the southernmost Western Ghats in India. *Acta Trop* 2017;171:64-7.
- [13] Bhattacharya SK, Dash AP. Elimination of Kala-Azar from the Southeast Asia Region. *Am J Trop Med Hyg* 2017;96(4):802-4.