Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2015; 3(3): 196-200
© 2015 JEZS
Received: 18-04-2015
Accepted: 20-05-2015

Vaishali R. Wabale
Assistant Professor, Department of Microbiology, Grant Government Medical College, Mumbai, Maharashtra, India.

Ameeta A. Joshi
Associate Professor, Department of Microbiology, Grant Government Medical College, Mumbai, Maharashtra, India.

Renu S. Bharadwaj
Professor & Head, Department of Microbiology, B. J. Medical College & Sassoon Hospital, Pune, Maharashtra, India.

Sarala G. Menon
Associate Professor, Department of Microbiology, Grant Government Medical College, Mumbai, Maharashtra, India.

Nilma N. Hirani
Assistant Professor, Department of Microbiology, Grant Government Medical College, Mumbai, Maharashtra, India.

Abhay S. Chowdhary
Professor & Head, Department of Microbiology, Grant Government Medical College, Mumbai, Maharashtra, India.

Correspondence:
Vaishali R. Wabale
Assistant Professor, Department of Microbiology, Grant Government Medical College & Sir J.J. Hospitals, Byculla, Mumbai- 400008, Maharashtra, India.

Post Kala Azar Dermal Leishmaniasis; A review of case series from Mumbai

Vaishali R. Wabale, Ameeta A. Joshi, Renu S. Bharadwaj, Sarala G. Menon, Nilma N. Hirani, Abhay S. Chowdhary

Abstract
Post Kala Azar Dermal Leishmaniasis (PKDL) is an unusual dermatosis caused by a parasite *Leishmania donovani* (LD). It occurs between six months and five years of following an attack of Visceral Leishmaniasis (VL) / Kala Azar (KA). As a recurrence of VL, it keeps appearing on the skin of patient even after 20 years of partial treatment, inadequate treatment and in untreated cases. We here 4 cases of PKDL in Mumbai a non- endemic zone of the country. In first case, laboratory diagnosis was made by demonstration of LD bodies on histopathological examination. In second and third case, diagnosis was made by detecting anti-leishmanial antibodies. In the fourth case, diagnosis was made by both demonstrations of LD bodies as well as anti-leishmanial antibodies. The PKDL lesions are confusing with the other dermatological conditions and hence, it is necessary that clinicians and pathologists should collaborate in diagnosing them so appropriate timely therapy can be instituted.

Keywords: Post Kala Azar Dermal Leishmaniasis (PKDL), Visceral Leishmaniasis (VL), Diagnosis, Treatment.

1. Introduction
Post Kala Azar Dermal Leishmaniasis (PKDL) is an unusual dermatosis caused by a parasite *Leishmania donovani* (LD). Untreated cases of PKDL are considered to be the sole reservoir to house and disseminate the causative parasite LD in the absence of zoonotic transmission [1]. *Phlebotomous argentipes*, the vector transmitting Visceral Leishmaniasis (VL) in India, when allowed to feed on PKDL patients, became infected and developed promastigote in the midgut, seeming capable of transmitting the parasite. It is not known whether the parasite in PKDL lesions is the residual parasite after VL infection or is introduced upon re-infection by sand fly vector [2].

PKDL manifests in 5-15 % of cases between 1 to 20 years after the cure of VL. Clinically, it is restricted to macular, papular or nodular lesions in the skin [3]. The disease manifests in a variety of clinical forms from hypo pigmented macules, erythematous or infiltrated, juicy papules to nodules and plaques. They are usually present on the face, limbs and trunk usually appearing in that order or all possible combinations of these [2, 4, 5].

Kala Azar (KA) is endemic in the North- eastern states of India like Bihar, West Bengal, and Assam and Plains of eastern Uttar Pradesh [4]. However PKDL was first described by Brahmachari in 1922 from Bengal, in cured VL patients manifesting with eruptions and plaques in the skin and named as 'dermal leishmanoid' [2]. LD bodies were observed in slit smears, hence, the condition was renamed as 'PKDL', since the eruptions follow KA. The pathogenesis of PKDL is obscure and there is no information about the association of PKDL with particular Zymodeme patterns. In India, *Leishmania* isolated from PKDL patients were found to have minicircle DNA absent from patients in KA [1].

Accurate diagnosis of PKDL is important due to the long and toxic treatment with antileishmanial drugs Antimony therapy for PKDL patients' needs to be continued for much longer duration than for VL patients (4 months instead of 4 weeks of VL). Treatment may last up to 6 months and the drugs can have serious side-effects for patients and is a waste of medical and economic resources. Therefore, a sensitive and specific protocol for diagnosis of PKDL is important.

Development of PKDL has been observed in patients who have been treated with Sodium Stibogluconate (SSG) and Pentamidine, either as irregular/incomplete or even after a full

course of therapy [6]. Some reports suggested that the incidence of antimony refractions in VL patients is due to the anthroponotic transmission of refractory strains from PKDL patients thus increasing the burden of drug resistance [7]. In India, the PKDL patients are considered as a reservoir of parasite that plays an important role in inter epidemic periods of VL. Hence, for the VL control, reliable diagnostic tests for the detection of PKDL is of utmost importance [5]. Though, the mortality from PKDL is low, the disease has a social stigma and carries a significant socioeconomic burden, which get further precipitated by noncompliance or a reluctance to obtain treatment [3].

The first step in the diagnosis of PKDL is assessing clinical signs and symptoms, and in endemic areas it is often diagnosed on clinical symptoms alone. However, there is a large geographical variation in clinical presentation and they can be confused with other skin disorders [8].

In this study, we have reported 4 cases of PKDL from Mumbai; a capital of the state of Maharashtra, India, a non-endemic area for Leishmaniasis makes this unusual.

2. Materials and Methods: The Grant Government Medical College (GGMC) and Sir J. J. Hospitals is situated in the south Mumbai and caters primarily to all the population of Mumbai city. The study was conducted in the Department of Microbiology, GGMC over a period of 03 years from January 2011 to December 2013. Here we report 4 cases of PKDL in non-endemic area of Mumbai, Maharashtra. These patients were clinically diagnosed as PKDL after attending Sir J. J. Hospitals Dermatology Out Patient Department (OPD) were studied. All 4 patients were migrants from known endemic states of the country. They were asked for the past history of dermatological diseases, KA, or any family history of KA. First patient was a 42 year old male from Bihar, working as a cart puller in Mumbai. He gave a history of KA in 1988, treated for it 20 years back. Second patient was a 22 year old male from Uttar Pradesh. He gave no past history of any major illness. Third patient was a 37 year old male from West Bengal. No history of KA was given by the patient. Fourth patient was a 12 year old female, studying in the 7th standard in Mumbai, from Jharkhand state (Godda district,) She gave a history of suffering from KA 6 years back which was treated at her native place. There, biopsy was done from a lesion on a leg. She was started with Tablet Hanseperan (50mg) once and Tablet Rifampicin (300mg) once daily for 45 days. She was diagnosed as Borderline Lepromatous Leprosy (BLL), and was treated for it 1 year back. The lesions kept recurring.

A general clinical examination was performed. [Table-1] A skin biopsy smear, Slit Skin Smear (SSS) and Anti-leishmanial antibodies were tested in the patient's serum using a rapid, visual spot test against a patented recombinant antigen (rKE16) [Flow Through, Anti-Leishmanial Spot/ Immunodot Test Kit; Span Diagnostics Ltd. India). [Table-2] Plain blood sample was cultured in Novy- Mac Neal- Nicolle (NNN) Modified Twin pack Medium (HI MEDIA Laboratories, Mumbai). All patients were treated with SSG (30ml IP equivalent to 100mg Pentavalent Antimony in each ml/vial) intramuscularly in each buttock for 30 days along with Amphotericin B and antibiotics. Human Immune Deficiency Virus (HIV) test was performed in all patients.

Table-1: Shows the Clinical Presentation and Differential Diagnosis.

Serial No.	Clinical Presentation	Differential Diagnosis
Case 1	*Hypopigmented patches on glans penis as well as on right shoulder	Known case of KA since 20 years
Case 2	*Light colored spots on face and hands which gradually increased in size, disappeared and fresh spots appeared. Sometimes these spots discharged a transparent fluid. *On examination: Few, soft, well-defined, discrete skin colored papules were present over the cheeks and hands.	Cutaneous Leishmaniasis (CL),Cutaneous Histoplasmosis (CH) and Borderline Tuberculoid Leprosy (BTL)
Case 3	*Light colored patches over face and body. *On examination: Hypopigmented patches were present over face, neck and back.	Clinically suspected as KA
Case 4	* Light colored red lesion over the chin, forearms and legs. The lesions eventually faded in 15 days leaving white color rash. New lesions kept appearing. *On examination: Erythematous, shiny papules present on the chin, ear pinna and upper lip region associated with erythema. Few red, papulovesicular lesions on the chin and perioral area. *Multiple, hypopigmented macules in present over face, hands [Fig-1] and depigmented macules on the trunk [Fig-2] coalescing to form large patches on the thigh. *Submandibular Lymph Nodes (LN) enlarged with palpable, non-tender axillary LN.	Borderline Lepromatous Leprosy (BLL) Histopathological diagnosis: - Borderline Tuberculoid Leprosy

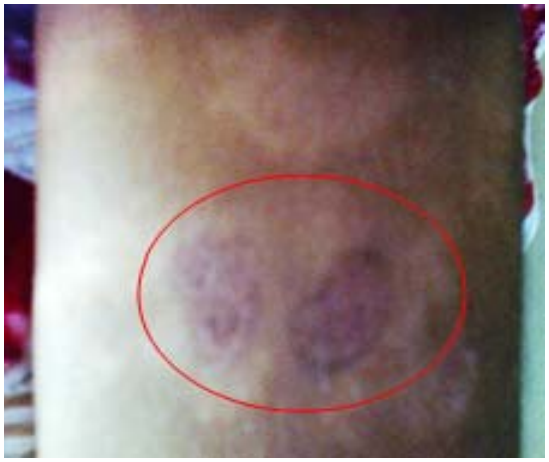

Fig 1: Hypopigmented macules on hands

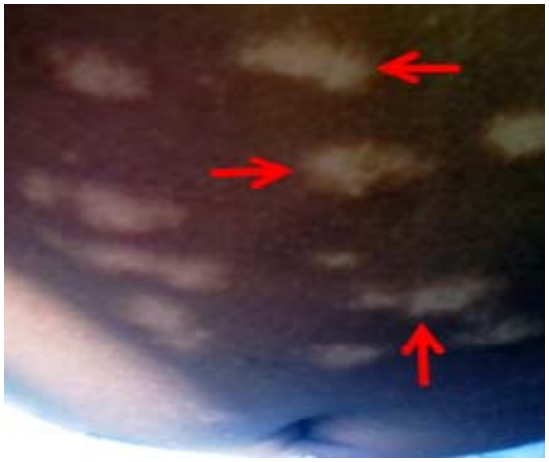

Fig 2: Depigmented macules on trunk

Table 2: Shows the Results of Microscopy Vis a Vis Anti-leishmanial Antibodies

Serial No.	Microscopic examination [Giemsa staining]	Anti-leishmanial Antibody detection [Flow Through, Anti-Leishmanial Spot Test]
Case 1	Skin biopsy smear: **positive** for scanty amastigote forms of LD bodies [Fig-3]	Patient's Serum: negative for anti-leishmanial antibody detection
Case 2	SSS examination : negative for amastigote forms of LD bodies	Patient's Serum: **positive** for anti-leishmanial antibody detection [Fig-4]
Case 3	SSS examination: negative for amastigote forms of LD bodies	Patient's Serum: **positive** for anti-leishmanial antibody detection
Case 4	SSS examination: **positive** for occasional amastigote forms of LD bodies [Fig-5]	Patient's Serum: **positive** for anti-leishmanial antibody detection

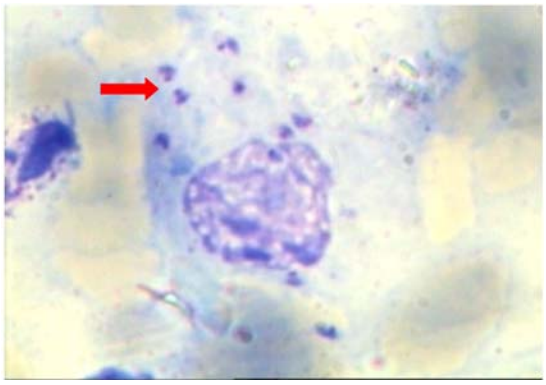

Fig 3: Skin biopsy smear showing amastigote forms of LD bodies (Giemsa stain, 1000X)

Fig 4: Anti-leishmanial antibody positive by 'Flow Through Anti-Leishmanial Spot' test

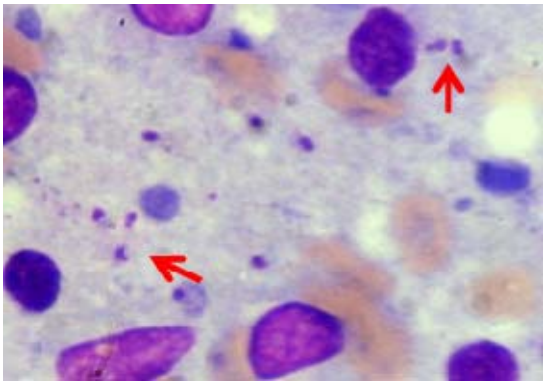

Fig 5: Skin Slit Smear showing occasional amastigote forms of LD bodies (Giemsa stain, 1000X)

3. Results

In our 1st and 4th case, the diagnosis of PKDL was made on the basis of SSS microscopy examination, which showed occasional amastigotes forms. In 2nd and 3rd case, diagnosis of PKDL was considered only after rKE16 antibody test came positive as the microscopy was negative. In the 1st case where rKE16 antibody test was negative but, scanty amastigotes were detected by SSS. In 2nd, 3rd and in 4th cases, rKE16 antibody test was positive. However, in the 4th case, both microscopy as well as rKE16 antibody tests were positive. In all the four cases, parasite failed to grow in cultured NNN medium.

4. Discussion

Leishmaniases refers to a group of infectious diseases caused by different species of protozoan parasites belonging to the genus *Leishmania* of the family *Trypanosomatidae*. In 1903, Leishman and Donovan discovered these parasites. As per the geographical location, these species has been classified as *L. donovani* in Asia, Africa and *L. infantum* in the Europe. Total six principal forms have been described on the basis of clinical and morphological characteristics are; *L. tropica*, *L. aethiopiana*, *L. donovani*, *L. braziliensis*, *L. infantum* and *L. chagasi* [1].

L. tropica causes Oriental Sore also known as CL of the Old World; *L. braziliensis* causes Mucocutaneous Leishmaniasis (MCL) of the New World and *L.* donovani causes VL which is commonly called as Dum Dum fever or KA [9]. Leishmaniasis usually categorized in 3 major forms depending on their severity of spontaneously healing skin ulcers found in CL and destructive MCL to fatal VL [2]. In the eastern regions of the India, VL is endemic, however often becomes epidemic and results into severe morbidity and mortality [10]. Leishmaniasis remains a major public health problem, affecting life of 12 million individuals, threatening 350 million and having a prevalence in 88 countries [2]. Every year approximately 2.5 million new cases get diagnosed with an estimated disease burden around 2.4 million disability-adjusted years. As a result, World Health Organization (WHO) has classified *Leishmaniasis* as a category-1 disease, an emerging and/or uncontrolled disease, acknowledging it as a severely neglected condition and urging the establishment of intensified research programs to improve vector control, diagnostics and therapeutic arsenal to contain further morbidity and mortality [11]. The geographical distribution is restricted to tropical and temperate regions according to the living area of the sand fly. The causative agent LD, is a flagellated promastigote, commonly found in its vector however, the non-motile, intracellular amastigote form resides within macrophages of human host.

Both VL and PKDL cases are caused by the same causative agent LD. The exact mechanism and etiopathogenesis of PKDL is not known yet [8]. So far, little is known about the factors of parasite/host origin that drive the parasite to cause a shift in the site of infection from viscera to dermis and thereby the clinical manifestations of the disease [2].

The extent of people who develop PKDL after infection without the development of VL has not been documented **(12)**. However, More than 90% of world's cases are found in India, Bangladesh, Brazil, Nepal and Sudan [13]. In India, different clinical forms of leishmaniasis such as CL, KA/VL and PKDL occur in the different geographic regions. CL occurs mostly in the north-western desert and semi-arid regions of state of Rajasthan. While cases have been reported from the hilly regions of Western Ghats of Kerala and Assam. A case of disseminated CL was reported from Chandigarh. In India, KA has its home in the plains of the Ganges and Brahmaputra. It

occurs endemically and epidemically in the eastern region, mainly Assam, West Bengal (9 districts), Bihar (30 districts), eastern districts of Uttar Pradesh, Jharkhand, foothills of Sikkim, Himalayas and Kashmir, and to a lesser extent in Tamil Nadu and Orissa, Gujrat [13].

KA is mostly restricted to the plains and does occur in altitudes above 2000 feet. In 1958 and 1964, KA and CL declined drastically following massive insecticide spaying campaigns for malaria eradication. However, the incidence started rising gradually in the late 1960s and early 1970s. The reason behind exposure of more and more people into vector prone areas is government run developmental projects, large water resources schemes along with colonization and resettlement programs.

PKDL is a late complication of KA, usually follows VL treatment. It occurs within 2 years of completion of therapy with SSG or after apparent cure of VL [1, 4]. It has been reported to occur in 5% of cases in India [4, 14]. The figure is much higher (20%) following African KA [4]. Occasionally, PKDL may develop during treatment of KA and should more appropriately be referred as 'Para-Kala Azar Dermal Leishmaniasis' [1]. In areas where KA is caused by LD, PKDL has been reported with a variable frequency or to an unknown extent [1]. It has been said that PKDL cases may act as a human reservoir of *Leishmania* parasite, but to what extent is unknown [1]. The pathogenesis of PKDL is obscure and the literature on it is sparse [15].

Particularly PKDL is a nodular form, the parasites are few in number and not always proportional to the extent of dermal lesions. While, it's dermal form maybe easily confused with a number of dermatological conditions such as vitiligo and leprosy [2, 16]. It may resemble leprosy clinically and pathologically, but loss of sensation, thickened nerves and other features are lacking. The occurrence of occasional neuritis in the lesions of PKDL could be another possible source of confusion between leprosy and KA [1]. Parenteral Amphotericin B has emerged as a very effective treatment for VL and the incidences of PKDL have also decreased after this therapy [13].

Currently, the diagnosis of PKDL is based on clinical and epidemiological parameters [2]. Demonstration of parasite in SSS/biopsy skin tissue is considered to be the gold standard for the diagnosis of PKDL. But these methods are invasive, less sensitive (58%) and difficult to perform at the field/periphery level [2, 13]. Other currently used methods for the diagnosis of VL are Enzyme Linked Immunosorbent Assay (ELISA) based serological tests, Leishmania Skin Test (LST) and Cultures on NNN medium. However, ELISA may be positive due to the past occurrence of VL and LST may or may not be positive in PKDL. The NNN Cultures are often not positive in PKDL and has a sensitivity of 54% only [2].

In hypo pigmented lesions of PKDL, LD bodies are usually absent in biopsy specimens making it difficult to diagnose [13]. When the parasite is not demonstrated in skin biopsies, the diagnosis of the PKDL depends on the endemicity of VL in the area and previous history of infection by the parasite [2]. Absence of VL history in 15-20% of PKDL suggests subclinical infection and makes diagnosis difficult [2].

The standard diagnostic approach at a tertiary, secondary or even primary health levels in areas of endemicity is microscopy, since sophisticated techniques are currently expensive and out of reach of general population [14]. Conventional diagnosis using histopathology in PKDL tissue sections using Haematoxylin and Eosin (H and E) staining shows a variable degree of positivity for LD bodies ranging from 67-100% in nodular lesions, 36-69% in popular lesions & 7-33% in macular lesions. Even in ideal situations, the success rate for LD bodies' demonstration is about 58% [2]. Confirmation of the diagnosis is usually done by SSS microscopy or histopathology. However, the reported sensitivity of SSS microscopy is, at best, 40-60% from patients with macular lesions and even lower in patients with macular lesions. In addition, parasite load between different clinical presentations may differ, which may mean that some diagnostic tests are more suitable for some patients than others. The advantage of microscopy is the acknowledged high specificity, which leads to low numbers of patients unnecessarily treated with anti-leishmanial drugs. However, because of the below par sensitivity many centers do not use this test [8].

The serological tests, such as Direct Agglutination Test (DAT) and ELISA for PKDL diagnosis are rapid and non-invasive tests. The interval between VL and PKDL in India is usually long, hence the immune response is likely to be the results of PKDL occurrence and not the persistence of antibodies of earlier VL infection [2]. Immunological tests provide useful alternative as rapid and sensitive measures for PKDL diagnosis; however, the question whether anti-leishmanial antibodies are these persisting due to VL or elicited due to PKDL remains unanswered [5].

Newer molecular methods like Polymerase Chain Reaction (PCR) are being developed for clinical diagnosis, and has proved to be promising [2]. In spite of the fact that there are many reports about the association of VL and Acquired Immune Deficiency Syndrome (AIDS), PKDL is very uncommon in Human Immune deficiency Virus (HIV) positive patients, and so far, only four cases have been documented in the literature. None of our patients had HIV co-infection. Though, SSG is the drug of choice in the treatment of VL and PKDL, it has shown a high relapse rate or failed to achieve a good response in PKDL. Amphotericin B has been used for a considerable length of time with a high efficacy rate [10]. All our 4 patients received SSG with the other drugs, responded well to the treatment; on follow-up, declared as free from PKDL. The disease may be under reported as clinical signs and symptoms are not specific and may not be suspected in areas which are not known to be endemic for KA. Increasing awareness of the disease will lead to better diagnosis and an appropriate management.

To conclude, it is important to diagnose all cases of PKDL as they are a major reservoir of infection in the community. An increasing awareness will lead to the clinician, to attempt a laboratory confirmed diagnosis by demonstration of LD bodies in SSS; demonstrating antibodies against the parasite or where facilities are available by PCR. An accurate diagnosis will go further in institution of appropriate therapy and thus control of *Leishmania* infection in the community.

5. References

1. Kordofani YM, Nour YT, El-Hassan AM, Shalayel MH. Post kala azar dermal leishmaniasis in Sudan. East Mediterr Health J. PubMed PMID: 15332749 2001; 7(6):1061-4.
2. Salotra P, Singh R. Challenges in the diagnosis of post kala-azar dermal leishmaniasis. Indian J Med Res. PubMed PMID: 16778312 2006; 123(3):295-310.
3. Mukhopadhyay D, Dalton JE, Kaye PM, Chatterjee M. Post kala-azar dermal leishmaniasis: an unresolved mystery. Trends Parasitol. PubMed PMID: 24388776; PubMed Central PMCID: PMC3919212 2014; 30(2):65-74.
4. Joshi A, Gulati A, Pathak VP, Bansal R. Post-Kala-Azar-

dermal-leishmaniasis: an unusual presentation from Uttarachal (a non-endemic hilly region of India). Indian J Dermatol Venereol Leprol. PubMed PMID: 17656928 2002; 68(3):171-3.
5. Saha S, Mazumdar T, Anam K, Ravindran R, Bairagi B, Saha B *et al*. Leishmania promastigote membrane antigen-based enzyme-linked immunosorbent assay and immunoblotting for differential diagnosis of Indian post-kala-azar dermal leishmaniasis. J Clin Microbiol. PubMed PMID: 15750095; PubMed Central PMCID: PMC1081224. 2005; 43(3):1269-77.
6. Das VN, Pandey K, Verma N, Lal CS, Bimal S, Topno RK *et al*. Short report: Development of post-kala-azar dermal leishmaniasis (PKDL) in miltefosine-treated visceral leishmaniasis. Am J Trop Med Hyg. PubMed PMID: 19270277 2009; 80(3):336-8.
7. Musa AM, Khalil EA, Younis BM, Elfaki ME, Elamin MY, Adam AO *et al*. Treatment-based strategy for the management of post-kala-azar dermal leishmaniasis patients in the Sudan. J Trop Med. PubMed PMID: 23690794; PubMed Central PMCID: PMC3649346 2013; 2013:708391.
8. Adams ER, Versteeg I, Leeflang MM. Systematic Review into Diagnostics for Post-Kala-Azar Dermal Leishmaniasis (PKDL). J Trop Med. PubMed PMID: 23935641; PubMed Central PMCID: PMC3723149 2013; 2013:150746.
9. El Hassan AM, El Hassan LA. Recent advances in visceral leishmaniasis. Alpa Adna microbiology journal 1997; 6:23-33.
10. Bora D. Epidemiology of visceral leishmaniasis in India. Natl Med J India. PubMed PMID: 10416321 1999; 12(2):62-8.
11. Man's DR, Zeegelaar JE, Hu RV, Kent AD, Ramdas S, Schalling HD. Clinical, biological, and anthropological aspects of leishmaniasis in Suriname - report of the final meeting of the Integrated Program, accessed 30 August, 2014. <https://www.academia.edu/7808483/Clinical_biological_and_anthropological_aspects_of_leishmaniasis_in_Suriname_-_report_of_the_final_meeting_of_the_Integrated_Program.
12. Siriwardana HV, Noyes HA, Beeching NJ, Chance ML, Karunaweera ND, Bates PA. Leishmania donovani and cutaneous leishmaniasis, Sri Lanka. Emerg Infect Dis. PubMed PMID: 17552105; PubMed Central PMCID: PMC2725894 2007; 13(3):476-8.
13. Koley S, Mandal RK, Choudhary S, Bandyopadhyay A. Post-kala-azar dermal leishmaniasis developing in miltefosine-treated visceral leishmaniasis. Indian J Dermatol. PubMed PMID: 23723490; PubMed Central PMCID: PMC3667302 2013; 58(3):241.
14. Wabale VR, Joshi AA, Bharadwaj RS, Chowdhary AS. Leishmaniasis: An Emerging Disease in Mumbai, Maharashtra, India. Research and Reviews: Journal of Medical and Health Sciences 2014; 3(1):77-82.
15. Ramesh V, Singh R, Salotra P. Short communication: post-kala-azar dermal leishmaniasis--an appraisal. Trop Med Int Health. PubMed PMID: 17596251. 2007; 12(7):848-51.
16. Osman OF, Oskam L, Kroon NC, Schoone GJ, Khalil ET, El-Hassan AM *et al*. Use of PCR for diagnosis of post-kala-azar dermal leishmaniasis. J Clin Microbiol 1998; 36(6):1621-4.