Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/q5576r89

CHALLENGES IN THE DIAGNOSIS OF POST-KALA-AZAR DERMAL LEISHMANIASIS A TRICKSTER DISEASE: CROSS-SECTIONAL STUDY AND AN OVERVIEW

Fariha Kauser[1*], Anshoo Agarwal[2], Nida Suhail [3], Kabir Singal[4], Harmanjeet Singh[5], Rashad Othman[6], Wajid Ali Chatha [7], Raghad Qasim Alshalan[8] , Fajer Atallah[9]

[1*]Health practitioner & Ex Medical Educationist from University of Dundee, Scotland
[2]Northern Border University, Kingdom of Saudi Arabia
[3]Department of Medical Laboratory Technology, Faculty of Applied Medical Sciences, Northern Border University, Arar, Saudi Arabia
[4]JSS Medical College , Mysore, Karnataka, India
[5]Mahatma Gandhi Memorial Medical College, Jamshedpur
[6]Northern Border University, Kingdom of Saudi Arabia
[7]Faculty of Medicine, Northern Border University, Arar, Kingdom of Saudi Arabia
[8]Medical student, Northern Border University, Arar, Kingdom of Saudi Arabia
[9]Medical student, Northern Border University, Arar, Kingdom of Saudi Arabia

***Corresponding Author:** Fariha Kauser
[*]Health practitioner & Ex Medical Educationist from University of Dundee, Scotland

Abstract:
Background Post-kala-azar dermal leishmaniasis (PKDL) manifests as a skin eruption after healing of visceral leishmaniasis (VL), either spontaneously or as a result of treatment. Tropical and subtropical areas are home to the disease leishmaniasis, which is brought on by the intracellular parasite. It is spread to people via the bite of the sand fly, Phlebotomus orientalis. Over all continents, the great majority of tropical regions have this infectious disease. On the other hand, the Middle East, Southeast Mexico, Central and South America, Northeastern Africa, Southern Europe, and South Asia exhibit greater case incidences. Leishmania donovani is a species of the subgenus Leishmania that mostly develops in the midgut and foregut of Phlebotomus orientalis, a kind of sand fly .

Material & Methods : We studied 100 cases of PKDL . The diagnosis was based on clinical presentation, positive slit skin smear and histopathologic studies. Clinical features were analysed and cytological and histopathological correlation was done.

Results: There were 64 (64%) males and 56 (56%) females. Generalized lymphadenopathy was present in seven cases. Slit skin smears revealed Leishman−Donovan bodies (LDBs) in 78 (78%) cases. Biopsy specimens revealed LDBs in 38(38%) cases only. Fine needle aspiration from cervical lymph nodes in 6(6%) cases demonstrated LDBs.

Conclusions: High positivity for LDBs were seen on slit smear examination of lesions. This study emphasizes the need to be aware that though the biopsy is gold standard for final diagnosis of the lesion, LDBs were detected more in the slit smears(78%) as compared to detection of LDBs in 38% cases on histopathology of PKDL cases . Hence slit smear examination may be more useful as compared to histopathology in PKDL cases.

Introduction:

Leishmaniasis is a protozoan disease whose diverse clinical manifestations are dependent both on the infecting species of Leishmania and the immune response of the host[1]. Transmission of the disease occurs by the bite of a sand fly infected with Leishmania parasites[2]. The distinctive histologic feature of this 2−5 μm parasite is the presence of both a nucleus and a smaller rod-shaped structure consisting of mitochondrial DNA called the kinetoplast and is called as "Leishmania donovani" [3]. Other names of leishmaniasis include Oriental sore, Aleppo evil, Delhi boil, Baghdad sore, Rose of Jericho, Chiclero's ulcer, uta, espundia (mucous form), forest yaws, Dumdum fever (visceral form), kala-azar, and black fever. The increases in travel and the number of immunocompromised individuals allows leishmaniasis to be considered an "emerging disease"[4]. Infection may be restricted to the skin in cutaneous leishmaniasis, limited to the mucous membranes in mucosal leishmaniasis, or spread internally in visceral leishmaniasis or kala azar. In the last two decades, leishmaniasis, especially visceral leishmaniasis, has been recognized as an opportunistic disease in the immunocompromised, particularly in patients infected with human immunodeficiency virus. Post-kala-azar dermal leishmaniasis which is a known complication of visceral leishmaniasis (VL) [5]. Usually, the infection is zoonotic. Humans are commonly an accidental host, although there are situations, such as visceral leishmaniasis (kala-azar) and PKDL, in which humans are the reservoir in an anthroponotic cycle[1]. Human leishmaniasis is classified as cutaneous (CL) or visceral (VL), but the species that cause visceral disease may also cause cutaneous lesions, as in the case of visceral leishmaniasis and PKDL[1].

Kala-azar (KA) is endemic in many parts of the world. The age distribution of PKDL in South Asia and Sudan differs, as in the former, young adults are more affected whereas in the latter, children are more affected [4]. A lag period ranging from 2 to 10 years exists between cure from VL and onset of PKDL, suggesting that PKDL echoes the epidemic of VL and can persist well after the epidemic. PKDL is limited to two geographically isolated regions: East Africa, namely Sudan [2], and South Asia, which includes India, Nepal, and Bangladesh [3]. While papular or nodular lesions are seen in the Sudanese version, polymorphic lesions—the coexistence of macules/patches and papulonodules—are more common in the South Asian variant[5]. Despite having a low death rate, PKDL is a stigmatizing illness with a heavy socioeconomic cost that is exacerbated by resistance to treatment or noncompliance. Because of the parasite-rich lesions, particularly the papulonodules, there is conjecture that PKDL is essential to the inter-epidemic spread of VL[6].

Detection of LDBs on histopathological examination of PKDL cases is low making final diagnosis difficult and also the histological findings in PKDL cases mimic many other skin lesions causing diagnostic difficulties[7]. Post kala-azar dermal leishmaniasis, which typically develops as a sequela after visceral leishmaniasis appears to have been successfully cured though it may be the most intriguing manifestation of leishmaniasis from a clinical and scientific standpoint. This study was undertaken to describe the clinical, and histopathologic features of PKDL and to see the usefulness of slit smear over histopathology in PKDL cases[8].

Material and methods:

Analyses of clinically suspected 100 cases of PKDL were done in the health centers of India and Pakistan. Histopathology and slit smear examination were done in all the cases. Slit skin smear aspirates from different types of lesion was performed and stained by Giemsa stain. Skin biopsy from the representative lesions was carried out for histopathologic examination. Tissue was subjected to Hematoxylin and Eosin and Giemsa stain. Fine needle aspiration of lymph nodes was performed in 13 cases.

Results:

A total number of 100 cases of PKDL were studied. There were 64 (64%) males and 56 (56%). The age ranged from 4 to 84 years and the majority (86.5%) were in the 10−30-year age group (Table1). The interval between KA and the appearance of PKDL ranged from 1 to 17 years with

mean interval being 3 years. Past history of kala-azar was present in 40(80%) cases. Clinically diagnosis of PKDL was considered in 80(80%) cases (Table 2). Fine needle aspiration from cervical lymph nodes in 6(6%) cases demonstrated LDBs.

S.No	Age group (year)	Gender		Frequency (%)	
		Male (%)	Female (%)	Total	%
1	< 10	16(16)	4(4)	12	12
2	10−20	24 (24)	22 (22)	46	46
3	21−30	16 (16)	14 (14)	30	30
4	31−40	4 (4)	2(2)	6	6
5	> 40	4 (4)	2(2)	6	6

Majority (86.5%) of the cases were in the 10−30-year age group

Table 2 Clinical diagnosis of PKDL cases.

S.No	Clinical Diagnosis	No of cases	Percentage (%)
1	PKDL	80	80
2	Lepromatous leprosy	2	2
3	Tuberculoid leprosy	2	2
4	Fungal infection	2	2
5	Tuberculosis	2	2
6	Foreign body granuloma	2	2
7	Malignant ulcer	2	2
8	No clinical diagnosis	2	2
	Total number of cases	100	100

Clinically diagnosis of PKDL was considered in 80(80%) cases.

Table 4: Site of PKDL lesions

S.No	Site of lesions	No of cases	Percentage (%)
1	Face	38	38
2	Ears	20	20
3	Arms	15	15
4	Scalp	5	5
5	Chest	1	1
6	legs	3	3
7	Abdomen	1	1
8	Site not mentioned	7	7
	Total number of cases	100	100

Majority of the lesions in PKDL were found on the face, ears and arms

Discussions:

PKDL manifests as a skin eruption after healing of VL, either spontaneously or as a result of treatment. It is considered to be an important reservoir for this parasite [1]. The eruption starts as hypopigmented macules 1−2 years after VL and the lesions gradually evolve into nodules [1-3]. Cases of CL acquired by among native citizens returning from endemic areashave been reported but without mucosal involvement[4-5]. PKDL has also been seen in patients without any history of VL similar to as was found in our study[6]. Usually, more than one type of lesion is present[7-8], only one type of lesion, such as hypopigmented macules, has been found to occur in one in 10 patients [9-10]. Initially, the lesions start as pin-point macules over different parts of the body and later grow and coalesce to form figurate lesions [11]. This explains the generalized and symmetrical nature of the disease [12]. The lesions can be localized, generalized, or extensive, localized lesions usually involve the face [13]. Generalized hypopigmented macules involving the trunk and extremities with erythema are the commonest presentation [13]. In the present study also similar

findings were observed. We have also recorded the pure macular type of PKDL in the same ratio as observed by others [9,11,13]. It has been suggested that the waist line is spared because of continuous pressure by clothing [14]. Nodular and noduloulcerative lesions involving the skin[15,16], nasal, oral, oropharyngeal, and laryngeal mucosa[17] , and glans penis [18], have been observed. Studies have shown that lesions of PKDL consistently appears on sun-exposed areas[18], particularly the face, ears, arms, etc., rather than unexposed areas, such as the scalp and chest which was concurrent with our findings. This supports the concept that exposure to UV light plays a contributory role in the pathogenesis of PKD[18]. Lymphadenopathy without visceral involvement in PKDL has been described[1]. One case of PKDL in our study had cervical and epitrochlear lympadenopathy without visceral involvement. Large study [19] done earlier have shown, the male to female ratio and age incidence similar to that observed in our study. Most of our patients were 10−30 years of age, as described by others [2] , Similar to other reports[9-11] , we also observed a history of KA in most patients. The clinical features revealed that most patients showed polymorphic lesions [7,8] such as macules, papules, plaques, and nodules distributed symmetrically and in generalized manner [12,13] , whereas a few showed extensive [13] or limited facial [13] involvement. In most studies [7, 8], polymorphic lesions have been described. Sandfly vectors responsible for visceral leishmaniasis (VL),cutaneous leishmaniasis(CL)and mucocutaneous leishmaniasis(ML) are present in certain areas of Asian countries[3].

Differentiation from leprosy is difficult in places where both diseases are endemic. Slit skin smears from the nodules, macules and plaques on the face, trunk, foot, leg and ear lobes for *Mycobacteriuum leprae* were negative, however, many *Leishmania donovani* bodies (LDB) were seen in these lesions. Bone marrow aspirate stained also showed many LDB. However, diagnosis of PKDL can be made clinically provided that the appearance, distribution and temporal relationship with kala-azar are taken into account[18]. Diagnosis can be supported by slit skin smear or biopsy examination for LDB. Therefore, it is important that physicians be aware of the fact that leprosy is an important differential diagnosis of PKDL[1] .

In PKDL patients, lymph nodes may be enlarged but most PKDL patients do not have demonstrable parasite in lymph node or bone marrow aspiration[20-21].

The histopathologic picture in macular lesions has been described in the literature [13,23]. A mixed chronic inflammatory cell infiltrate of histiocytes, lymphocytes, and a few plasma cells has been observed in the upper dermis in macular lesions[13,23]. Leishman−Donovan bodies (LDBs) [9,12,23] , are seen in small numbers. In the absence of LDBs, it is difficult to make a definite diagnosis of PKDL. Nevertheless, a history of KA, clinical features, and response to treatment can help in making a diagnosis In nodular lesions, the infiltrate is seen in the entire dermis. In the case of papules and plaques, typical histopathology may allow a definite diagnosis of PKDL even in the absence of LDBs[15]. Histopathologic studies reveal epidermal and/or dermal changes, depending on the type and stage of the disease [24]. The diagnostic histopathologic changes of leishmaniasis, however, are usually present in the dermis. There is a predominantly mononuclear dermal infiltrate consisting primarily of lymphocytes and histiocytes. The histiocytes may be filled with Leishman-Donovan (L-D) bodies, which are 2−4 μm oval encapsulated protozoa with a large peripheral nucleus and a smaller rod-shaped kinetoplast of mitochondrial DNA. L-D bodies are numerous in early lesions of LCL and PKDL, very abundant in DCL, but scanty in MCL, VL, and leishmaniasisv recidivans, an unusual form of the disease not discussed here in detail[25-27].

Immunity after cure of KA has been known to be almost lifelong, provided there is no immunosuppression[28]. A degree of immunosuppression induced by intercurrent diseases such as measles, malaria, tuberculosis and HIV infections favors reinvasion of the parasites from the skin to the viscera[29].

Post kala-azar dermal leishmaniasis (PKDL) as the name suggests, develops usually after 1−2 years of treated kala-azar(KA) [30-32]. 7(14%) of PKDL in our study occurred after more than 10 years of treated Kala azar . Such a long disease-free interval may be forgotten by the patient, and if not carefully elicited in the history, the diagnosis of PKDL may be missed. Skin-slit smears showed

Leishman-Donovan bodies in these cases. Skin biopsy revealed infiltration of the entire dermis by lymphocytes, histiocytes and ill-defined epithelioid cell granulomas. Diseases like syphilis, fungal diseases [33], sarcoidosis, and lymphoproliferative[34]disorders, which can present a similar histopathologic picture can make diagnosis of PKDL on histopathology difficult in such cases. There was a wide variation in the duration of PKDL and in the interval between KA and PKDL. Moreover, there was no correlation between the interval and the type of lesion (it has been suggested that initially the lesions start as macules and later evolve into papules and plaques[1-11].
Serologic assays have been described recently, including the recombinant Leishmania donovani gene B protein (rGBP) enzyme-linked immunoabsorbent assay (ELISA)
[35-37]. Traditionally, the accepted methods for diagnosis have been microscopy and culture. It is possible to do a punch or wedge biopsy. Dermal cells extracted with a root canal file, scalpel scrapings, slit skin smears, and excised tissue can all be processed with a touch preparation or Giemsa-stained tissue impression slide.
The identification of the specific species of parasite responsible for infection is important for the diagnosis of disease, evaluation of therapy, and prognosis. Consequently, a number of molecular biology techniques have been developed that are designed for species-specific identification of parasites within the genus *Leishmania*.
Patients who get appropriate therapy typically begin to improve within a week, and full hematological recovery takes place between four and six weeks of treatment [4]. Relapses are uncommon, and the patient is deemed treated if none are discovered within six months of follow-up [5,35].
Visceral leishmaniasis is difficult to diagnose due to a number of reasons, such as its subtle onset, protracted febrile state, signs and symptoms that might be confused with those of typhoid and malaria, and endemic areas that lack access to modern medical facilities. Testing can be extremely difficult due to the disease's complex presentation, as most endemic places with high disease burdens are unfortunately impoverished [38]. Furthermore, the symptoms and indicators of visceral leishmaniasis can be ambiguous and deceptive, necessitating a thorough laboratory examination to rule out other probable causes. Frequent medical visits are also necessary due to the visceral disease's hidden and chronic nature. The gold standard for diagnosis and confirmation is bone marrow aspiration, which necessitates sophisticated hospital setups with qualified doctors and adds to the budgetary burden.

Conclusions:

KA is an intriguing protozoal illness with a wide range of pathologic and clinical presentations. The last factors that support the diagnosis of PKDL are the prior history of KA, the nonulcerative character of the nodules, the histopathology's exclusion of LL, and the positive therapeutic response to antimony injections. Clinical diagnosis of PKDL can be challenging at times, and even with gold-standard skin biopsy histology, LDBs may not always be demonstrable, and results may resemble those of other illnesses. In situations of PKDL, slit smear examination may be quite helpful in identifying LDBs and ultimately aiding in the final diagnosis.

References

1. Leishmaniasis: a review. Torres-Guerrero E, Quintanilla-Cedillo MR, Ruiz-Esmenjaud J, Arenas R. F1000Res. 2017;6:750.
2. Leishmaniasis. Piscopo TV, Mallia Azzopardi C. Postgrad Med J. 2007;83:649–657
3. A review of leishmaniasis: current knowledge and future directions. Mann S, Frasca K, Scherrer S, Henao-Martínez AF, Newman S, Ramanan P, Suarez JA. https://doi.org/10.1007/s40475-021-00232-7. Curr Trop Med Rep. 2021;8:121–132.
4. Ganguly S, Saha P, Chatterjee M, Roy S, Ghosh TK, Guha SK, *et al.* PKDL – A silent parasite pool for transmission of leishmaniasis in kala-azar endemic areas of Malda District, West Bengal, India. PLoS Negl Trop Dis 2015;9:e0004138.

5. Verma N, Bimal S, Das VN, Pandey K, Singh D, Lal CS,*et al*. Clinico pathological and immunological changes in Indian post kala-azar dermal leishmaniasis (PKDL) cases in relation to treatment: A retrospective study. Biomed Res Int 2015;745062:1-5.
6. Hematologic changes in visceral leishmaniasis/kala azar. Varma N, Naseem S. Indian J Hematol Blood Transfus. 2010;26:78–82.
7. Visceral leishmaniasis in a resident of Swat, Khyber Pakhtunkhwa, Pakistan, presenting to Civil Hospital Karachi: a case report. Saifullah Khan S, Shahab MH, Naz T. Cureus. 2019;11:0.
8. "Kala-azar is a dishonest disease": community perspectives on access barriers to visceral leishmaniasis (kala-azar) diagnosis and care in Southern Gadarif, Sudan. Sunyoto T, Adam GK, Atia AM, et al. Am J Trop Med Hyg. 2018;98:1091–1101 1.
9.Hossain F, Picado A, Owen SI, Ghosh P, Chowdhury R, Maruf S, et al. Evaluation of Loopamp™ Leishmania Detection Kit and Leishmania Antigen ELISA for Post-Elimination Detection and Management of Visceral Leishmaniasis in Bangladesh. *Front Cell Infect Microbiol* [Internet]. 2021. Apr 26;11:670759. Available from: https://pubmed.ncbi.nlm.nih.gov/33981632 [PMC free article]
10. World Health Organisation. Leishmaniasis [Internet]. 2021. https://www.who.int/news-room/fact-sheets/detail/leishmaniasis
11. Mondal D, Nasrin KN, Huda MM, Kabir M, Hossain MS, Kroeger A, et al. Enhanced Case Detection and Improved Diagnosis of PKDL in a Kala-azar-Endemic Area of Bangladesh. *PLoS Negl Trop Dis* . 2010. Oct 5;4(10):e832. Available from: doi: 10.1371/journal.pntd.0000832
12. Zijlstra EE, Musa AM, Khalil EAG, el-Hassan IM, el-Hassan AM. Post-kala-azar dermal leishmaniasis. *Lancet Infect Dis*. 2003. Feb;3(2):87–98. doi: 10.1016/s1473-3099(03)00517-6
13. Singh A, Ramesh V, Ramam M. Histopathological characteristics of PKDL: a series of 88 patients. Indian J Dermatol Venereol Leprol 2015;81:29-34.
14. Samuel LP, Balada-Llasat JM, Harrington A, Cavagnolo R.Correction for Samuel *et al.*, Multicenter assessment of gram stain error rates. J Clin Microbiol 2016;54:2405.
15. Sarraf NR, Mukhopadhyay S, Banerjee A, Das AK, Roy S, Chakrabarti S, et al. Genome wide comparison of Leishmania donovani strains from Indian visceral Leishmaniasis and para-kala-azar dermal leishmaniasis patients. *Acta Trop* [Internet]. 2021;223:106086.
16. Chapman LAC, Spencer SEF, Pollington TM, Jewell CP, Mondal D, Alvar J, et al. Inferring transmission trees to guide targeting of interventions against visceral Leishmaniasis and post-kala-azar dermal Leishmaniasis. 2020;117(41).
17. Kumar R, Das VNR, Topno RK, Pal B, Imam A, Agrawal K, et al. Para-kala-azar dermal Leishmaniasis cases in Indian subcontinent—A case series. *Pathog Glob Health* [Internet]. 2016;110(7–8):326–9. Available from: doi: 10.1080/20477724.2016.1258163
18. Kumar P V., Sadeghi E, Torabi S. Kala azar with disseminated dermal Leishmaniasis. *Am J Trop Med Hyg*. 1989;40(2):150–3. doi: 10.4269/ajtmh.1989.40.150
19. Sarker CB, Alam KS, Jamal MF, Rahman S, Huq MH, Musa AK, et al. Sensitivity of splenic and bone marrow aspirate study for diagnosis of kala-azar. *Mymensingh Med J*. 2004. Jul;13(2):130–3.
20. Moulik S, Sengupta S, Chatterjee M. Molecular Tracking of the Leishmania Parasite. *Front Cell Infect Microbiol* [Internet]. 2021. Feb 22;11:623437. Available from: https://pubmed.ncbi.nlm.nih.gov/33692966 doi: 10.3389/fcimb.2021.623437
21. Ghosh P, Hasnain MG, Hossain F, Khan MAA, Chowdhury R, Faisal K, et al. Evaluation of Real-time PCR for Diagnosis of Post-Kala-azar Dermal Leishmaniasis in Endemic Foci of Bangladesh. *Open forum Infect Dis* [Internet]. 2018. Sep 15;5(10):ofy234–ofy234. Available from: https://pubmed.ncbi.nlm.nih.gov/30320150 doi: 10.1093/ofid/ofy234 .
22. Salam MA, Mondal D, Kabir M, Ekram ARMS, Haque R. PCR for diagnosis and assessment of cure in kala-azar patients in Bangladesh. *Acta Trop*. 2010. Jan;113(1):52–5.
23. Ghosh P, Sharma A, Bhattarai NR, Abhishek K, Nisansala T, Kumar A, et al. A Multi-Country, Single-Blinded, Phase 2 Study to Evaluate a Point-of-Need System for Rapid Detection of

Leishmaniasis and Its Implementation in Endemic Settings. 2021; Available from: doi: 10.3390/microorganisms9030588
24. Ministry of Health and Family Welfare Bangladesh. National Guideline For Kala-azar Case Management.2013;(Cdc):1–72. http://kalacorebd.com/wp-content/uploads/2016/04/KA-Managment-Guideline-18-05-2013_v10-1.pdf
25. Saravolatz LD, Bern C, Adler-Moore J, Berenguer J, Boelaert M, den Boer M, et al. Liposomal Amphotericin B for the Treatment of Visceral Leishmaniasis. *Clin Infect Dis* [Internet]. 2006. Oct 1;43(7):917–24. Available from: doi: 10.1086/507530
26. Lima H, Vasconceles W, David JR, Lerner EA: American cutaneous leishmaniasis: In *situ* characterization of the cellular immune response over time. *Am J Trop Med Hyg* 1994;50:743−747.
27. Modlin RL, Pirmez C, Hofman FM, et al: Lymphocytes bearing antigen-specific gd T-cell receptors accumulate in human infectious disease lesions. *Nature* 1989;339:544−548.
28. EL-Hassan AN, Ghalib HW, Zijlstra EE, *et al.* Post kala-azar dermal leishmaniasis in the Sudan: clinical features, pathology and treatment. *Trans R Soc Trop Med Hyg* 1992; 86:245−248.
29. Nandy A, Addy M, Maji AK, *et al.* Recurrence of kala-azar after PKDL: role of co-factors. *Trop Med Int Health* 1998; 3: 76−78.
30. Action HW, Napier LE. Post-kala-azar dermal leishmaniasis. *Ind Med Gaz* 1929; 64: 147−148.
31. Napier LE, Das Gupta CR. A clinical study of Post-kala-azar dermal leishmaniasis. *Ind Med Gaz* 1930; 65: 249−257.
32. Thakur CP. Epidemiological, clinical and therapeutic features of Bihar Kala-azar (including Post-kala-azar dermal leishmaniasis) *Trans Roy Trop Med Hyg* 1984; 78: 391−398.
33. Hobbs RE, Hempstead RW. Cutaneous coccidioidomycosis simulating lepromatous leprosy. *Int J Dermatol* 1984; 23: 334−336.
34. Singh K, Dawar R, Ramesh V. lymphocytic infiltration of skin of Jessner-Kanof masquerading as borderline leprosy. *Ind J Lepr* 1985; 57: 804−806.
35. Jensen, AT, Gasim, S, Moller, T *et al.* Serodiagnosis of *Leishmania donovani* infections: assessment of enzyme linked immunosorbent assays using recombinant *L. donovani* gene B protein (GBP) and a peptide sequence of *L. donovani* GBP . *Trans R Soc Trop Med Hyg* 1999; 93, 157 160.
36. Gasim, S, Elhassan, AM, Khalil, EA *et al.* High levels of plasma IL-10 and expression of IL-10 by keratinocytes during visceral leishmaniasis predict subsequent development of Post-kala-azar dermal leishmaniasis. *Clin Exp Immunol* 1998; 111, 64 69.
37. Osman, OF, Oskam, L, Kroon, NC *et al.* Use of PCR for diagnosis post-kala-azar dermal leishmaniasis. *J Clin Microbiol* 1998; 36, 1621 1624.
38. Uzun S, Uslular C, Yucel A *et al.* Cutaneous leishmaniasis: evaluation of 3074 cases in the Cukurova region of Turkey. *Br J Dermatol* 1999; 140: 347 50.