Symposium
Dermatopathology

Histopathological features in leprosy, post-kala-azar dermal leishmaniasis, and cutaneous leishmaniasis

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ABSTRACT

Leprosy, cutaneous leishmaniasis, and post-kala-azar dermal leishmaniasis are common infectious diseases, the latter two being seen mainly in endemic areas. With increased migration within the country, these diseases are now frequently being seen in major cities. This brief review article focused mainly on histopathology will be useful for the dermatologists and pathologists to be familiar with the basic histopathology of these lesions.

Key words: Cutaneous leishmaniasis, histopathology, leprosy, post-kala-azar dermal leishmaniasis

INTRODUCTION

Leprosy is a chronic infectious disease caused by *Mycobacterium leprae* (*M. leprae*) and is predominantly a disease of skin and peripheral nerves with or without systemic involvement. Pathogenesis of leprosy is complex and its clinicopathological manifestations are the result of host-parasite interactions.[1,2]

Despite its falling prevalence rate, it continues to be a cause of significant public health problem in endemic regions. Globally, 211903 new leprosy cases were detected in 2010.[3] The most affected countries are India and Brazil with some countries in Sub-Saharan Africa and Southeast Asia.[4] The mode of transmission is still unknown, but it is believed to be through inhalation of bacilli that are excreted from the nasal passages of the multibacillary patient. Direct person-to-person transmission through skin contact can occur as lepra bacilli can survive in favorable environmental conditions for long duration. There have also been isolated reports of its transmission from hypodermic needles during skin tattooing or by physical trauma to skin. The present article gives a brief review of histopathological characteristics of leprosy.

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IMMUNOPATHOGENESIS OF LEPROSY

The sequence of disease pathogenesis in leprosy is complex and depends on the host-parasite immunological responses. Leprosy is the classical example of the disease with an immunopathologic spectrum wherein the host immune reaction to the infective agent ranges from none to marked with a consequent range of clinicopathologic manifestations. Tuberculoid leprosy (TT) shows a high cellular response characterized by T-cell and macrophage activation and very few bacilli in the tissues. Lepromatous leprosy (LL) on the opposite pole shows an absent cellular immune response to *M. leprae* antigens with no macrophage activation and abundant bacilli in the tissues. The immunopathologic spectrum is a dynamic continuum, in which the patients move in either direction according to the host immune response

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and treatment. The standard delineation follows the classification of Ridley and Jopling[1] with categories defined along this spectrum by a combination of clinical, microbiological, and histopathological indices: Tuberculoid (TT), borderline tuberculoid (BT), midborderline (BB), borderline lepromatous (BL), and lepromatous leprosy (LL). The TT and LL group of patients are stable, the former often self-healing and the latter remaining heavily infected unless given chemotherapy. The central point of the spectrum BB is most unstable with patients quickly downgrading to LL if not treated. Apart from these, there are some patients who are labeled as 'indeterminate' leprosy and these are the patients with the earliest identifiable skin lesions that cannot be categorized definitely in any immunopathologic spectrum.

HISTOPATHOLOGY OF LEPROSY

Biopsy from a well-developed cutaneous lesion is an important procedure for diagnosis and classification of leprosy. Standard histopathological examination of the formalin-fixed paraffin-embedded skin tissue can provide information regarding cellular morphology, presence of acid fast bacilli (AFB), and can be enhanced by techniques like immunohistochemistry and molecular studies. Histopathological examination of the skin biopsy from a leprosy patient helps to; (a) confirm the diagnosis of leprosy; (b) classify the disease in the leprosy spectrum; (c) identify the bacillary load in the tissue; (d) assess disease activity and response to treatment; (e) confirm and classify lepra reactions.

Indeterminate leprosy

Indeterminate leprosy is the earliest detectable skin lesion comprising one or few hypopigmented macules with no clear sensory changes. The skin biopsy may show mild accumulation of lymphocytes and macrophages and an occasional AFB either in the non-inflamed nerve, arrector pili or in the sub-epidermal zone in the very early stages. It may show neuritis evidenced by Schwann cell proliferation and infiltration of the nerve fibers with lymphocytes. Nerve infiltration is the most significant feature of leprosy when the rest of the skin shows non-specific changes. Moreover, the histological changes are known to precede the clinical manifestations by at least few months.[5] Most indeterminate leprosy cases are known to heal spontaneously,[6] but since it is not possible to predict which indeterminate cases will evolve into well-known forms, it is ethical to treat all the patients.

Tuberculoid leprosy

Primary polar tuberculoid leprosy has large and compact epithelioid cell granulomas along the neurovascular bundles with lymphocytes. Langhans giant cells are typically scanty or absent, and AFB are rare to find. Epithelioid cell granulomas always erode into the basal layer of the epidermis. The dermal nerves may be either obliterated and completely effaced or eroded by lymphocytes.

Borderline tuberculoid leprosy

The epithelioid granulomas of BT do not invade into the epidermis and have less lymphocytes in comparison to TT [Figure 1a]. The granulomas are arranged in a curvilinear pattern along the neurovascular bundle. Nerve erosion by the granuloma is typical, and AFB are scanty (ranging from bacteriological index (BI) 0-2) and are more readily detected in the Schwann cells of the nerves. In addition to nerves, the granuloma can also involve the sweat glands and the arrector pili muscle.

Mid-borderline leprosy

The histopathology in BB shows almost equal admixture of epithelioid cells and macrophages forming a distinct granuloma. The lymphocytes are scant and scattered and multinucleate giant cells are absent, a feature that helps it to be distinguished from BT. AFB may be frequent (ranging from BI 2-4).

Borderline lepromatous leprosy

The predominant cells in the granulomas are macrophages with occasional epithelioid cells arranged in patches [Figure 1b]. Lymphocytes are sparse, AFB are abundant (ranging from BI 4-5) but usually not present as globi. Perineural fibroblast proliferation forming 'onion-skin' in cross section is a typical feature. Foamy histiocytes are frequently seen.

Lepromatous leprosy

The typical features consist of a flattened epidermis separated from the dermal infiltrate by a grenz zone of normal collagen also called as band of Unna. The macrophage granuloma of LL is large and expansile one consisting of sheets of histiocytes with only few lymphocytes [Figure 1c]. The histiocytes harbor abundant AFB (BI 5-6). The solid bacilli are stacked like cigars and appear as globi [Figure 2d]. Such an appearance is the rule rather than an exception. In

contrast to tuberculoid leprosy, the nerves in the skin of LL patients may contain considerable AFB; however, the morphological features of the nerve are fairly well preserved in the earlier phase of the disease before eventually becoming fibrotic. Presence of foamy change in LL suggests regression.

Pure neuritic leprosy

Pure neuritic leprosy is characterized by neural involvement in the absence dermal lesions. The histopathological examination of a nerve reveals a granuloma or infiltrate characteristic of leprosy.[7]

Lucio leprosy

The histopathology of this Mexican variant is similar to LL but with a characteristic heavy bacillation of the small blood vessels of the skin, leading to thrombosis of vessels and ischemia and ulceration called as the 'lucio' phenomenon.

Histoid leprosy

This is another variant of LL, which shows the highest load (BI 6) of solid staining AFB arranged in clumps and sheaves. The macrophage reaction is unusual in the sense that the macrophages become spindle-shaped and oriented in a storiform pattern reminiscent of a fibrohistiocytoma.

HISTOPATHOLOGICAL DIFFERENTIAL DIAGNOSIS

Tuberculoid leprosy needs to be differentiated from other granulomatous dermatitides. Cutaneous tuberculosis is the most important differential diagnosis, which has to be excluded. The epidermis in tuberculoid leprosy is usually flat and not hyperplastic as in tuberculosis. The arrangement of the granulomas in leprosy is along the neurovascular bundles giving an oblong pattern to the granuloma unlike tuberculosis where there is intense and sometimes lichenoid pattern of the chronic granulomatous infiltrate. The dermal nerve twigs when seen are spared by the infiltrate in tuberculosis. The presence of granuloma or AFB in the nerve is a conclusive proof of leprosy. Cutaneous sarcoidosis may sometimes be confused with tuberculoid leprosy as fibrinoid necrosis may be found in both these entities. The granulomas of sarcoidosis show paucity of lymphocytes and are more confluent and show fibrosis around the granuloma. Other granulomatous lesions like leishmaniasis or granulomatous post-kala-azar dermal leishmaniasis also need to be excluded by demonstration of Leishman-Donovan bodies and frequent presence of plasma cells. Borderline lepromatous and pure lepromatous leprosy may be confused with histiocyte-rich lesions like xanthomas; however, demonstration of AFB in these lesions usually solves the diagnostic dilemma.

HISTOPATHOLOGY OF REACTIONS IN LEPROSY

Leprosy reactions are periodic episodes of acute inflammation caused by immune responses to *M. leprae* or its antigens superimposed on the chronic course of the disease. There are two main types of leprosy reactions depending on the immunological mechanism, i.e. type 1 lepra or reversal reaction and type 2 lepra reaction, which is an immune complex manifestation. In type 1 lepra reaction, the biopsy will show invasion of the epidermis by the granulomatous infiltrate [Figure 2a] and edema in the superficial dermis [Figure 2c]. The granuloma becomes more epithelioid, shows infiltration of lymphocytes within and around them, and the Langhans giant cells become increased in number and bigger in size and may also show bizarre shapes. The granulomas also erode into the epidermis representing the upgrading reaction. In addition, caseous necrosis and acid-fast bacilli may be seen in the nerves. The skin and nerves are infiltrated by an influx of CD4 lymphocytes and macrophages[8] that secrete an array of cytokines of Th1 class like interferon-γ and tumor necrosis factor-α and are responsible for the inflammation and tissue damage.[9] These changes of epidermal erosion, dermal edema, intragranuloma edema, and lymphocytes within the granuloma are clues favoring a diagnosis of leprosy type 1 reaction.

Type 2 lepra reaction is characterized by varying degree of polymorphonuclear infiltration superimposed on the already existing granuloma. Edema is frequently present in the dermis. Deposition of immune complexes in the small cutaneous capillaries, arterioles, and venules results in necrotizing vasculitis.[10] This type of reaction is also called as erythema nodosum leprosum and is reflected by deeper infiltration of foamy histiocytes into the subcutaneous fat and presence of neutrophils [Figure 2b]. The influx of neutrophils can be intense so as to form neutrophilic microabscess. The AFB are fragmented and granular. Superficial ulceration, bulla formation, and necrosis may sometimes supervene.

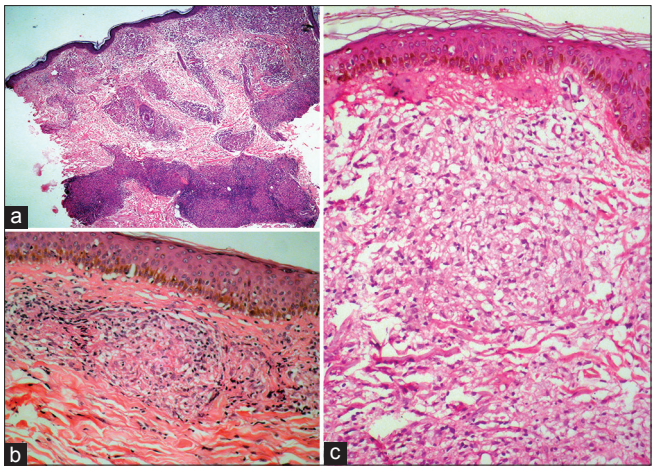

Figure 1: Photomicrograph showing (a) Compact epitheloid granulomas, (H and E, ×40), (b) Patchy perivascular collection of histiocytes and epitheloid cells, (c) Diffuse sheets of histiocytes separated by a sub-epidermal grenz zone (H and E, ×200)

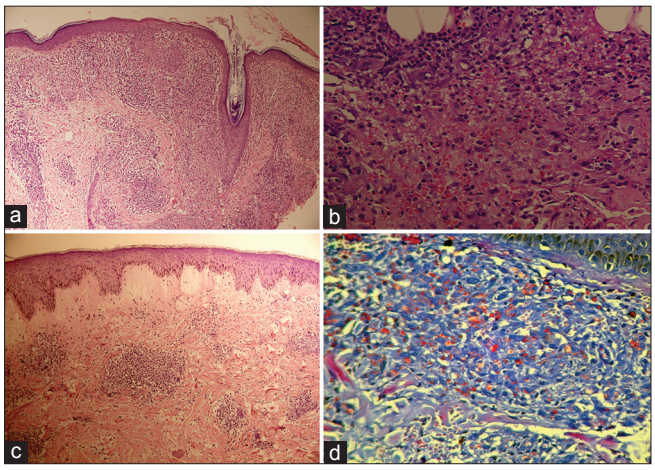

Figure 2: Photomicrograph showing (a) Granulomas eroding the epidermis, (b) Superficial dermal edema, (H and E, ×100), (c) Collections of neutrophils and histiocytes in fat lobule, (d) Fite stain showing numerous globi of AFB (H and E, ×400)

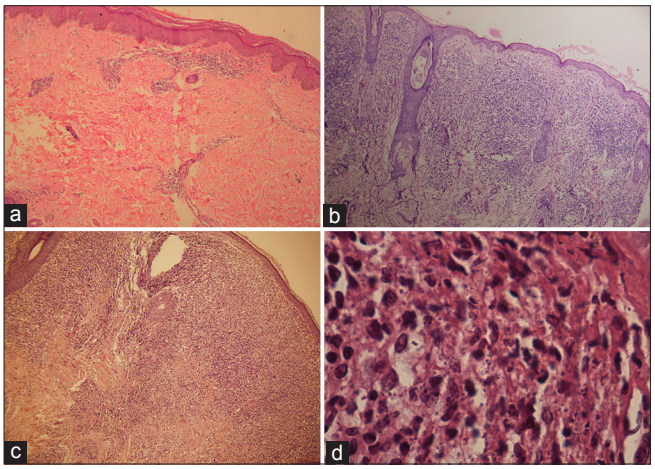

Figure 3: Photomicrograph showing (a) Superficial perivascular infiltrate, (b) Dense lymphohistiocytic infiltrate in upper dermis, (c) Follicular plugging and grenz zone, (H and E, ×100), (d) Intracytoplasmic Leishman-Donovan bodies (H and E, ×400)

To conclude, histopathological examination is integral to the understanding of leprosy, its causative organism and for monitoring relapse and drug resistance. Diagnosis of leprosy has been based on classical cardinal signs, characteristic histopathological findings, and demonstration of acid-fast bacilli both from the skin smears and skin biopsies of these lesions. The current primary goal is early diagnosis of this disease in order to interrupt the transmission by treating it early. As new serological and molecular tests become available for the early diagnosis of leprosy and its reactions, histopathological examination remains an integral tool for diagnosis and classification.

HISTOPATHOLOGY OF POST-KALA-AZAR DERMAL LEISHMANIASIS

Post-kala-azar dermal leishmanasis (PKDL) is an uncommon sequel seen in patients with previous attack of visceral leishmaniasis or kala azar (KA). It is caused by a protozoan *Leishmania donovani* and was first described from Bengal, India, in 1922.[11] The vector is a sandfly *Phlebotomus argentipes*, which feeds on these patients, becomes infected, and further transmits the disease to humans. Man is the only known vertebrate host of *L. donovani*, in India. PKDL is clinically characterized by hypopigmented macules, erythematous eruptions that gradually evolve to papules, plaques, and nodules.

PKDL is predominantly seen in East Africa and the Indian subcontinent. The frequency of PKDL following KA in Indian patients is from 5-10%[12] compared to 50% in Africa.[13] In Indian patients, the interval between occurrence of KA and PKDL ranges from 1-20 years (mean 6.2 years), which is longer as compared to African PKDL that usually develops within few months to an year.[14] In 15-20% of the cases, there is no previous history of KA,[12] but these patients invariably come from the KA endemic region.

This review discusses the histopathological characteristics of PKDL. Though hypopigmented macules appear early in PKDL, studies from endemic regions have shown that they usually occur in combination with other lesions. A purely macular presentation has been observed in about 10% of PKDL patients in Indian reports,[15,16] and in African PKDL, they constitute about 5% of all cases.[17] In a series of 14 pure macular PKDL, Ramesh and Singh[18] have shown three distinct clinical patterns of

macular PKDL; localized, generalized, and extensive. Clinically, the most important differential diagnosis of macular PKDL is leprosy. Since the parasites are scanty to absent in the macular variant, the diagnosis is usually based on history, clinical examination, exclusion of other diseases, and response to therapy. The biopsy from macular lesions almost always consists of sparse inflammatory infiltrate predominantly around the vessels of superficial vascular plexus [Figure 3a]. The inflammatory cells consist of lymphocytes, histiocytes, and few plasma cells. The main difficulty is confidently recognizing the organisms in this sparse infiltrate as it can be easily confused with karyorrhectic debris. When LD bodies are not present, the presence of plasma cells is an important clue in favor of PKDL. Unlike macular PKDL where the infiltrate is superficial, the macular lesions of leprosy show the inflammatory infiltrate centered around the neurovascular plexus in lower dermis.[19]

The most common clinical presentation of Indian PKDL is a polymorphic lesion that is a combination of macules, papules, and nodules. Irrespective of the clinical type of PKDL, the epidermis shows several changes in different combinations that include hyperkeratosis, acanthosis, atrophy with flattening of rete ridges, hydropic degeneration of the basal layer. Biopsy from the papules and plaques show a moderate to dense inflammatory cell infiltrate in the top half of the biopsy [Figure 3b]. Another important observation usually seen in papules and plaque lesions is the presence of follicular plugging and grenz zone, a strip of clear dermal collagen that separates epidermis from the infiltrate [Figure 3c]. Lymphocytes are the predominant cells when the infiltrate is moderate in intensity but as the density of infiltrate increases, the histiocytes and plasma cells are greater in number. Leishman-Donovan (LD) bodies are seen as intracytoplasmic structures in the cytoplasm of the histiocytes [Figure 3d].

Nodular PKDL takes a longer duration to develop, and the biopsy from the nodular lesions show a diffuse dermal inflammatory infiltrate consisting of histiocytes and plasma cells in large numbers. The epidermis overlying the infiltrate is stretched by the bulk of inflammatory cells. In contrast to lepromatous leprosy where the peripheral limits of the infiltrate are infiltrative, nodular PKDL have a fairly sharp margin. Since nodules most easily catch the physicians' eye, they are prone to be biopsied more frequently. Here, differentiation from lepromatous leprosy is of paramount importance. In a series of 26 nodular PKDL, Singh, *et al*.[20] reported that the epidermis in these biopsies was atrophic and the dermal adnexae were either caught up in the infiltrate or were displaced downwards. Compact epitheloid granulomas are seen more frequently in the nodules than in the macules or papules. In some situations like this when the LD bodies are numerous, their identity can seldom be confused with histoplasmosis. This can be easily resolved by doing fungal stains like PAS and silver methanamine. Other less common observations can be perineural infiltrate that can cause diagnostic difficulties between leprosy and PKDL.[21,22] Hyalinization in and around the vessel walls may also be seen, an observation reported by Singh, *et al*.[20] from a series of Indian PKDL patients.

Leishman-Donovan (LD) bodies if scanty are best found just beneath the epidermis, an observation reported by El-Hassan.[17] Sometimes, karyorrhexis may mimic LD bodies. In a study of 50 PKDL patients, Beena *et al*.[23] found LD bodies in half of the biopsies on the H and E stained sections. Special stains like Giemsa stain or iron hematoxylin do not have any additional advantage in staining the amastigotes. They further did immunohistochemical (IHC) detection of LD bodies by using G2D10, a mouse monoclonal antibody raised against a promastigote membrane antigen of *L. gerbelli*. This improved the detection rate to 80%. Another study from 30 Sudanese PKDL patients showed the detection rate of LD bodies on H and E stain as 17%, which increased to 88% when immunoperoxide staining using monoclonal antibody (D2) (2E5-A8) specific to *L. donovani* was performed.[24,25] Rathi, *et al*.[26] demonstrated LD bodies in 25% of patients with nodules and plaques and in none of the macules.

Beena, *et al*.[23] could not demonstrate any LD body on H and E stained slides of macular PKDL but could demonstrate them after IHC. In Indian PKDL patients, a remarkable affinity to genital skin and mucosa has also been observed.[27]

To conclude, PKDL can occur without previous history of KA, and since LD bodies may not be always demonstrable, familiarity with the spectrum of histopathological findings may suggest a clue to histopathologic diagnosis of PKDL in the absence of visualization of the parasites. When mucosa is

involved, biopsy should preferably be done from the mucosal lesion that is more likely to show the organisms.

HISTOPATHOLOGY OF CUTANEOUS LEISHMANIASIS

The histopathology of CL shows some parallels with those of other cutaneous infective granulomas. The variation in clinical and histopathological picture depends on the strain of the organism, the size of the inoculums, and the endemicity. Strains of leishmania organism also have variations in their genetically determined virulence. For instance, *L. tropica minor* in the Middle East usually results in a single dry ulcer, whereas *L. tropica major r*esults in multiple weeping ulcers.[28,29]

A recent report[30] describes four histopathological patterns ranging from a mixed inflammatory pattern composed of lymphocytes, plasma cells and macrophages with many LD bodies to a granulomatous pattern with few or no LD bodies. Another study from Himachal Pradesh, in which the lesions were caused by *L. donovani*, showed non-caseating epithelioid granuloma in 77% of the cases and LD bodies were demonstrable in 37% of tissue smears.[31] In another instance, *L. major* was identified in a case of CL mimicking lupus vulgaris.[32] In a study of 50 patients from Bikaner, Rajasthan, 64% of the biopsies were positive for LD bodies. (R.D. Bumb, personal communication) Hence, confirmation of the diagnosis is considerably improved when molecular methods like PCR are performed with both sensitivity and specificity exceeding 90%.[33] Since species other than *L. tropica* are assuming importance in CL, the practice of describing the parasites in tissue smear or histopathology as 'LT' bodies should be discouraged and the eponymous term 'LD' bodies is to be used, which is a general term for any of the species of the genus *Leishmania*.

REFERENCES

1. Ridley DS, Jopling WH. Classification of leprosy according to immunity: A five group system. Int J Lep 1966;34:255-73.
2. Ridley DS. Histological classification and the immunological spectrum of leprosy. Bull World Health Orga 1974;51:451-65.
3. World Health Organ. Global leprosy situation. Wkly Epidemiol Rec 2010;85:337-48.
4. Noordeen SK. Eliminating leprosy as a public health problem; why the optimism is justified. Int J Lep 1995;63:559-66.
5. Ridley DS. Classification of leprosy. In: *Window in leprosy.* Wardha: Gandhi Memorial Leproy foundation; 1978.
6. Crawford CL, Hardwicke PM, Evans DH, Evans EM. Granulomatous hypersensitivity induced by sensory peripheral nerve. Nature 1977;265:457-9.
7. Kumar B, Kaur I, Dogra S, Kumaran MS. Pure neuritic leprosy in India: An appraisal. Int J Lepr Other Mycobact Dis 2004;72:284-90.
8. Yamamura M, Uyemura K, Deans RJ, Weinberg K, Rea TH, Bloom PR, *et al*. Defining protective responses to pathogens: Cytokine profiles in leprosy lesions. Science 1991;254:277-9.
9. Sreenivasan P, Misra RS, Wilfred D, Nath I. Lepromatous patients show T helper1-like cytokine profile with differential expression of interleukin-10 during type 1 and 2 reactions. Immunol 1998;95:529-36.
10. Wemambu SN, Turk JL, Waters MF, Rees RJ. Erythema nodosum leprosum: A clinical manifeastation of the arthus phenomenon. Lancet 1969;2:933-5.
11. Brahmachari UN. A new form of cutaneous leishmaniasis-dermal leishmanoid. Indian Med Gaz 1927;57:125-7.
12. Mukherjee A, Ramesh V, Mishra RS. Post kala azar dermal leishmaniasis: A light and electron microscopic study of 18 cases. J Cutan Pathol 1993;20:320-25.
13. Musa AM, Khaleel EA, Raheem MA, Zijlstra EE, Ibrahim ME, Elhassan IM, *et al*. The natural history of Sudanese post kala azar dermal leishmaniasis: Clinical, immunological and prognostic features. Ann Trop Med Parasitol 2002;96:765-72.
14. Zijlstra EE, Musa AM, Khalil EAG, El-Hassan IM, El-Hassan AM. Post-kala-azar dermal leishmaniasis. The Lancet Infect Dis 2003;3:88-97.
15. Ramesh V, Misra RS, Saxena U, Mukherjee A. Post kala azar dermal leishmaniasis: A clinical and therapeutic study. Int J Dermatol 1993:32:272-5.
16. Girgla HS, Marsden RS, Singh GM, Ryan TJ. Post kala azar dermal leishmaniasis. Br J Dermatol 1977;97:307-9.
17. El Hassan, Ghalib HW, Zijlstra EE, Eltoum IA, Satti M, Ali MS, *et al*. Post kala azar dermal leishmaniasis in the Sudan-clinical features pathology and treatment. Trans R Soc Trop Med Hyg 1992:86:245-8.
18. Ramesh V, Singh N. A clinical and histopathological study of macular type of post kala azar dermal leishmaniasis. Trop Doc 1999;29:205-9.
19. Ismail A. Immune responses and immunopathology of post kala azar dermal leishmaniasis (PKDL) (PhD thesis) University of Copenhagen, Denmark: 1999.
20. Singh N, Ramesh V, Arora VK, Bhatia A, Kubba A, Ramam M. Nodular post kala azar dermal leishmaniasis: A distinct histopathological entity. J Cutan Pathol 1998;25:95-9.
21. Khandpur S, Ramam M, Sharma VK, Salotra P, Singh MK, Malhotra A. Nerve involvement in Indian post kala azar dermal leishmaniasis. Acta Derm Venereol 2003;84:245-6.
22. El Hassan AM, Ali MS, Zijlstra EE, Eltoum IA, Ghalib HW, Ahmed HM. Post kala azar dermal leishmaniasis in the Sudan: Peripheral neural involvement. Int J Dermatol 1992;31:400-3.
23. Beena KR, Ramesh V, Mukherjee A. Identification of parasite antigen, correlation of parasite density and inflammation in skin lesions of post kala azar dermal leishmaniasis. J Cutan Pathol 2003;30:616-20.
24. Ismail A, Gadir AF, Theander TG, Kharazami A, El Hassan AM. Pathology of post kala azar dermal leishmaniasis: A light microscopical, immunohistochemical and ultrastructural study of skin lesions and draining lymph nodes. J Cutan Pathol 2006;33:778-87.
25. Ismail A, Kharazami A, Permin H, El Hassan AM. Detection and characterization of *Leishmania* in tissues of patients with post kala azar dermal leishmaniasis using a specific monoclonal antibody. Trans R Soc Trop Med Hyg 1997;91:283-5.
26. Rathi SK, Pandhi RK, Chopra P, Khanna N. Post kala azar dermal leishmaniasis. Ind J Dermatol Venereol Leprol 2005;71:250-53.
27. Ramesh V, Mukherjee A. Post kala azar dermal leishmaniasis. Int J Dermatol 1995;34:85-91.
28. Rau RCC, Dubin HV, Taylor WB. Leishmania tropica: Infections in travelers. Arch Dermatol 1976;112:197-201.

29. Norton SA, Frankenburg S, Klaus SN. Cutaneous leishmaniasis acquired during military service in the middle east. Arch Dermatol 1992;128:83-7.
30. ul-Bari A, ber-Rahman A. Correlation of clinical, histopatholgical and microbiological findings in 60 cases of cutaneous leishmaniasis. Indian J Dermatol Venereol Leprol 2006;72:28-32.
31. Sharma NL, Mahajan VK, Kanga A, Sood A, Katoch VM, Mauricio I, *et al*. Localized cutaneous leishmaniasis due to *Leishmania* donovani and *Leishmania* tropica: Preliminary findings of the study of 161 new cases from a new endemic focus in Himachal Pradesh, India. Am J Trop Med Hyg 2005;72:819-24.
32. Kumar R, Ansari NA, Avninder S, Ramesh V, Salotra P. Cutaneous leishmaniasis in Nepal: Leishmania major as a cause. Trans R Soc Trop Med Hyg 2008;102:202-3.
33. Al-Hucheimi NS, Sultan BA, Al-Dhalimi AM. A comparative study of Old World cutaneous leishmaniasis in Iraq by polymerase chain reaction, microbiologic and histopathologic methods. Int J Dermatol 2009;48:404-8.