International Journal of PharmTech Research
CODEN (USA): IJPRIF, ISSN: 0974-4304, ISSN(Online): 2455-9563
Vol.9, No.12, pp 640-645, 2016

Some histopathological features of cutaneous leishmaniasis- in Iraqi patients

Israa K. Al-Aubaidi[1]*, Nagham Y. Al Bayati[2], Saddam. Al Haidari[1] and Muna H. Al-Azzawi[2]

[1]Department of biology , College of Education for Pure Science (Ibn Al-Haitham), University of Baghdad, Baghdad, Iraq
[2]Department of biology, College of Education for Pure Science, University of Diyala, Diyala, Iraq

Abstract : Cutaneous leishmaniasis is a global problem health in the world and Iraq. The present study was conducted to evaluate the clinical and histopathological features of cutaneous leishmaniasis (CL) in the skins of Iraqi patients that may help in the diagnosis of CL rather than the presence of Leishman-Donovan (LD) bodies in the infected human skin.Thirty-five biopsies were collected from individuals infected with CL during the period from October 2013 to April 2014. The clinical diagnosis was done and histopathological features of CL lesions were recorded. Skin biopsies were taken from the edges of the lesions and then fixed in 10% formalin, stained with hematoxyline and eosin stain and the histopathological findings were recorded and the results were analysed. The amastigotes of both *Leishmania tropica* and L. major were found in 88.57% of patients (62.85% males and 37.14% females). Other histological features that also help in the diagnosis of CL (even in the absence of LD bodies) were hyperkeratosis (23.07%), parakeratosis (19.23%), acanthosis (7.69%), pseudoepitheliomatous hyperplasia (10.25%), atrophy (12.82%), ulcer (17.94%) and crust (8.97%). In contrast, dermal inflammatory infiltrate of histocytes with amixture of lymphocytes, macrophages, plasma cells, giant cells and occasional eosinophils, neutrophils and mononuclear cells infiltrating the dermis were found with intra- and extra-cellular amastigotes. Other dermal reactions were a granulomatous inflammation and necrosis.This study suggests that the use ofhistopathological features of cutaneous leishmaniasis (CL) in the skinsmay help in the diagnosis of CL rather than the presence of Leishman-Donovan (LD) bodies in the infected human skin.
Key words : Cutaneonsleishmaniasis, Skin, Ulcer, Biopsy, Histopathological changes.

Introduction

Leishmaniasis is a group of vector borne diseases that are caused by obligate intracellular protozoan flagellate parasitesof the genus *Leishmania*, which are transmitted by the infected female *Phlebotomus* sand flies[1,2,3]. *Leishmania* parasites exist in two forms: Amastigote form which is an ovoid and non-flagellated, measuring 3-5 µm in length and found inside the macrophages of humans and other mammalian hosts, and promastigote from which is flagellated and found in the sandfly host [4]. There are mainly three clinical types of leishmaniasis, caused by various species of Leishmania, which are visceral, cutaneous and mucocutaneous[5].Theincidence of cutaneous leishmaniasis (CL) isestimated to be 1.0 – 1.5 million cases a year, and possesses a substantial risk for settlers, residents, military personnel and expatriates working or traveling to

endemic areas[6]. The causative agents of CL are *L. major*, *L.tropica*, *L. mexicana* and *L. amazonensis*[7,8]. In Iraq, two species of Leishmania which cause CL are present*; L. tropica*, the causative agent of anthroponotic cutaneous leishmaniasis (ACL), and *L. major*, the agent of zoonotic cutaneous leishmaniasis (ZCL) [9,10]. Both species are mainly endemic in different regions of Iraq [11].

The virulence of parasite, the host defense mechanism and some environmental factors determine the clinical features and the course of infection[12]. Cutaneous leishmaniasis begins as a nodule or papule and becomes a chronic ulcer that is restricted to the skin which is the most common form of CL[8,13,14]. Invasion of the skin leads to a tissue reactionthat may range from a mild inflammatory cell infiltrate with a large number of macrophages containing amastigotes to a highly organized and predominantly epithelial cell granuloma with very few or no demonstrable amastigotes[15]. The objective of this study was to evaluate the clinical and histopathological features of CL in Iraqi patients that can help in the diagnosis of CL rather than depending on the presence of LD bodies in the human skin.

Materials and Methods

The CL patients were Iraqi citizens, who had been admitted to the Dermatology Clinic at Baquba Teaching Hospital in Diyala Province during the period from October 2013 to April 2014. Thirty five male and female patients aged 5-65 years were included in this study. The clinical diagnosis was confirmed by laboratory demonstration of the amastigotes (LD bodies) in the stained cutaneous impression smears by light microscopic examination.

Different clinical lesions were noted such as plaque, nodule, crusted lesions and ulcers. None of these patients have received any kind of treatment before the biopsies have been taken. Skin biopsies were taken from the edges of the lesions (avoiding ulcerated parts) with 4mm disposable punch and 2% xylocaine as an anaesthetic. The biopsy material was preserved in 10% formalin, routinely processed and embedded in paraffin. Sections were cut and stained with eosin-haematoxylin.

Results

Histopathological changes that have been recorded included the presence or absence of hyperkeratosis , acanthosis, parakeratosis, pseudoepitheliomatous epidermalhyperplasia, necrosis, unorganized or organized granuloma, eosinophils, giantandpolymorphnuclears cells:lymphocytes, plasma cells, macrophages (histocytes) infiltration andpresenceof amastigotesof *Leishmania* parasites. A total of 35 patients were included in the present study. Among these patients, 22 (62.85%) were males and 13 (37.14%) were females. Their age ranged from 5 to 65 years (mean: 18.74 ± 14.57 years).

As far as the site of lesions is concerned, 9 lesions were recorded on hands (25.71%) and 8 (22.85%) on face, 7 lesion on arm (20%), 5 lesions on legs (14.28) and 6 lesions on feet (17.14%) (Figure 1.). The majority of lesions were found on the parts of extremities which are usually uncovered by clothing, such as the arms, the backs of the hands and the ankles. In 22 patients (62.85%) multiple lesions were observed (Figure 2).

Fig.1. A single lesion on left hand. Fig. 2. Multiple lesions on the face. Fig 3. Large numbers of *Leishmania tropica* amastigoste within the macrophages(Giemsa stain x100).

The size of lesions ranged from 1 to 3 cm. Leishman-Donovan (LD) bodies were seen in histopathology slides in 31 patients (88.57 %), they were seen as oval bodies with 3-4 µm in diameter with around nuclei surrounded by clear halo area (Figure 3). Kinetoplasts were frequently noted with Giemsa stain.

Different histopathological features were grouped as epidermal changes and dermal reactions, the epidermal changed were variable and included hyperkeratosis with parakeratosis (Figure 4). Both atrophy and epidermal acanthosis may approach pseudoepitheliomatous epidermal hyperplasia (Figure 5).

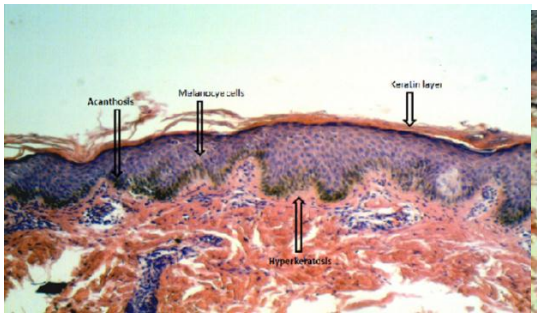


Fig. 4. Hyperkeratosis with features of parakeratosis, and Acanthosis and even pseudopitheliomatous hyperplasia (H & E. x40).

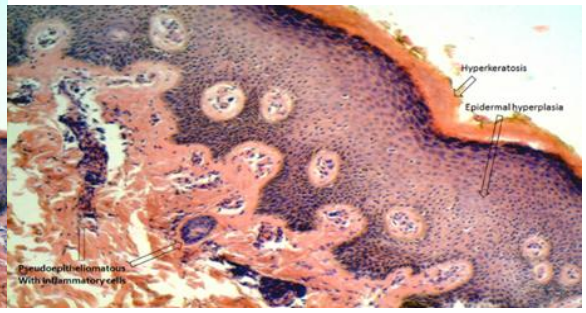


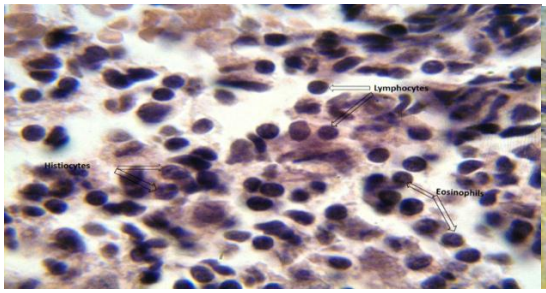
Fig.5.Hyperkeratosis,Epidermalhyperplasia Pseudoepitheliomatous with inflammatory (H & E. x40).

A hyperkeratosis was present in 18 cases, parakeratosis in 15 cases, acanthosis in 6, pseudoepitheliomatous hyperplasia in 8, epidermalatrophy in 10, ulceration in 14 and crust in 7 cases. Epidermal histopathological changes are shown in Table 1. Also follicular plugging is seen. It is rare to find *Leishmania* organisms presents within the epidermis.

Table 1. Epidermal histopathological changes of cutaneous leishmaniasis ulcers

Number of cases (%)	Epidermal histopathological
18 (23.07 %)	Hyperkeratosis
15 (19.23 %)	parakeratosis
6 (7.69 %)	Acanthosis
8 (10.25 %)	Psedoepitheliomatous hyperplasia
10 (12.82 %)	Epidermal atrophy
14 (17.94 %)	Ulceration
7 (8.97 %)	Crust
8 (10.25 %)	Amastigotes

In the dermis, the inflammation is dense to diffuse and massive infiltration is composed predominantly of histocytes with a mixture of lymphocytes. Plasma cell may be found primarily with in the macrophages and occasional eosinophil leukocytes were seen (Figures 6 and 7). Protozoal organisms are usually found within phagocytic cells but when they are numerous, they may also be seen extracellularly (Figure8).



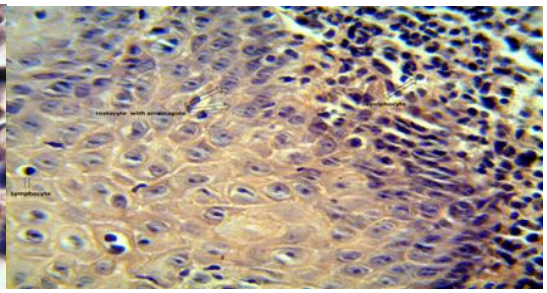


Fig. 6. Dermal infiltration consisting of lymphocytes, Fig.7. In dermis, infiltrate of histocytes and a few histocytes and eosinophil leukocyte (H & E x 40). lymphocytes within of the histocytes there are multiple small, round organisms and eccentric kinetoplasts (H & E. x1000).

When the infiltrate fills the dermis, there may be loss of the adnexal structures. Epithelial granulomas (granulomatus inflammatory response) are surrounded by mild to moderate infiltrate of histocytes and lymphocytes (Figure 8). Necrosis was also found as an area of necrosis which has been infiltrated by granulocytes, giving rise to microabscess (Figure 9).

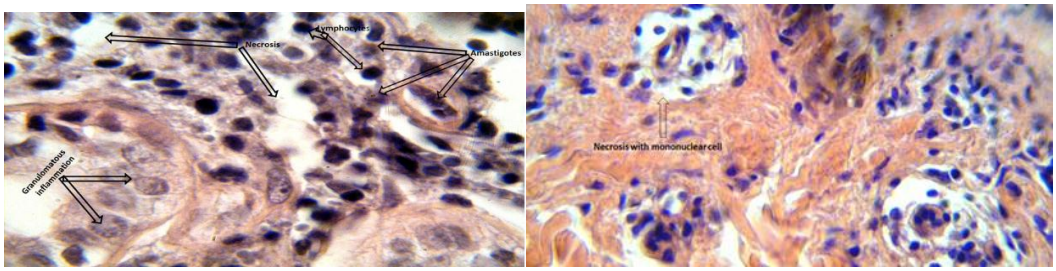


Fig. 8.Cutaneous leishmaniasis, skin,granulomatousFig. 9. Diffuse necrotic dermatitis with neutrophils inflammation, numerous amastigotes(H & E. x1000).and mononuclear cells infiltrating the dermis (H& E. x40).

Discussion

Cutaneous leishmaniasis (CL) is a major public health problem in many countries including Iraq[16]. Two species of Leishmania have been isolated in Iraq; *Leishmaniatropica* and *L. major* which are both implicated in the development of wet and dry lesions characterized by early ulceration and late ulceration, respectively [17].

The results of the present study showed that the prevalence of CL in males (62.85%) was significantly higher ($P< 0.05$) than in females (37.14%) and this may be due to the fact that the men work outdoors or sleep in open areas and may also due to the fact that men do not wear long clothes and cover themselves as in women so they are exposed to the bites of infected female sand flies [18]. Some studies have hypothesized that the gender difference observed in some parasitic diseases can be attributed to hormonal effects. However, controversy still exists regarding the role of sex hormones in the cellular immune response[19]. Although it is believed that sex hormones may influence the establishment and the course of parasitic diseases, the behavioral factors making males more likely to be exposed to the vectors in the fields and provide proper environments for transmission, are probably equally or more importantly in increasing the chances of exposure to the bites of infected female sand flies during their active hours [20,21]. On the contrary, other studies found higher incidence of infection among females than in males[22-25]. According to a prospective study conducted in Iraq in 2009[26], more CL cases occurred in males (57%) than in females (43%) and another study conducted in Islamabad (Turkey) in 2012 [27], also showed higher prevalence of infection in males (70%) than in females (30%).

Multiple and single cutaneous lesions were found on exposed areas of the bodies of the individuals included in the present study such as hands, faces, arms, legs, and feet. Similarly, some previous studies conducted in Iraq[5,6,26] and in Pakistan [27] have reported more or less similar observations.

After being bitten by an infected female sand fly, the nodules, papules or ulcers begin to develop and the amastigotes of the parasites begin to multiply within the macrophages and then naturally heal after leaving scars. Concerning the histopathological features, the present study found epidermal changes (hyperkeratosis with parakeratosis). Both atrophy and epidermal acanthosis may approach pseudoepitheliomatous epidermal hyperplasia. It is rare to find *Leishmania*amastigotes within the epidermis[28]. In a study conducted by Gumurduluet al.[29] in Southern Anatolia, Turkey, they reported that the lesions of CL were found on facial regions in 19 cases and histopathological changes with ulceration in 11 cases, epidermal atrophy in 9, acanthosis in 6, pseudoepitheliomatous hyperplasia in 8 and granulomas in 18 cases.

In the dermis, the inflammation is dense to diffuse with massive infiltration which is composed predominantly of histocytes with a mixture of lymphocytes. Plasma cell may be found primarily within the macrophages and occasionally eosinophil leukocytes were seen. It is well known that protozoal parasites are found within phagocytic cells but when these parasites increase in their numbers, they may also be seen extracellularly, where the infiltrate fills the dermis and may lead to the loss of the adnexal structures. Epithelial granulomas (granulomatus inflammatory response) surrounded by mild to moderate infiltrate of histocytes and

lymphocytes were seen. Necrosis was also found where areas were infiltrated by granulocytes, giving rise to micro abscesses. These observations are in agreement with those reported by Sharquie and Hameed [30] who studied two cases of CL lesions in Baghdad using histopathological examination of the margins of the ulcerative plaque and found ulceration of the epidermis, diffuse dense mononuclear cellular infiltration of lymphocytes, histiocytes and plenty of plasma cells throughout the dermis with early granuloma formation and they have also seen large numbers of amastigotes of *Leishmaniatropica* inside and outside the macrophages of the superficial dermis but there were fatty lobules with other changes where causing sepal and lobular panniculitis.

A granuloma is defined as a compact collection of mature mononuclear phagocytes and not necessarily accompanied by accessory features such as necrosis. Andrade-Narvaez etal.[31] reported that the essential features of CL pathology are the colonization by amastigote, cells of the mononuclear phagocytic system and the granulomatous inflammatory response. Moreover, Moravvejetal.[32] studied the cutaneous ulcers in Iranian patients and they recorded granulomatous in the dermis with central a cellular necrosis, histiocytes and lymphocytes aggregation in adjacent dermis.

References

1. Roberts, L.S. and Janovy, J. Jr. Foundations of parasitology, Seventh edition, The McGraw-Hill companies Higher education, Inc. London,2005.
2. Paniker, C.K. Textbook of medical parasitology, Sixth edithion, Jaypee brothers, New Delhi, India,2007,248P p.
3. World Health Organization. Control of the leishmaniasis. In proceeding of a meeting of the WHO, Expert committee, Ganeva, 2010, 22-26.
4. Rahi, A.A. Cutaneous leishmaniasis at Wasit Governorate. Baghdad Sci. J., 2011, 8, 286 – 288.
5. Rahi, A.A., Nsaif, S., Hassoni, J., Ali, M.A. and Hamza, H.A. Comparison of diagnostic methods in cutaneous leishmaniasis in Iraq, Am. J. Biosci.,2013, 1(1), 1 – 5.
6. El-Deen, L.D. Abul-Hab, J. and Abdulah, S.A. Clinico-epidemeiological study of cutaneous leishmaniasis in a sample of Iraqi Armed Forces. Iraqi J. Comm. Med.,2006, 19, 98-103.
7. Molan, A. L. and Faraj, A.M. Concise medical parasitology. First edition, Shabab printing house-Eerbil,Iraq.2010
8. Singh, S. New developments in n diagnosis of leishmaniasis. Ind. J. Med. Res.,2006, 123: 311 – 330.
9. World Health Organization. Communicable Disease working Group on Emergencies, HQ Division of communicable disease control, EMRO, WHO office, Baghdad, 2003, 39-44.
10. Al-Samarai, A.M. and Al-Obaidi, H.S. Cutaneous leishmaniasis in Iraq. J. Infect. Devel. Count., 2007, 3(2),123 - 129.
11. World Health Organization. Report of the consultative meeting on cutaneous leishmaniasis, Neglected tropical disease, The WHO document production services, Geneva, Switzerland,2007, 1-31. 12-
12. World Health Organization. Manual for case management of cutaneous leishmaniasis in WHO-Eastern Mediterranean region, Regional office for the Eastern Mediterranean, Cairo, Egypt,2014,7 – 48.
13. Maheshwari, N. Clinical microbiology and parasitology, 2nd edition, Jaypee Brothers Medical,LTD., New Delhi-India,2008,310pp.
14. Masmoudi, A.; Hariz, W. Marrekch,S. Amouri, M. and Turki, H. Old World cutaneous leishmaniasis: diagnosis and treatment. J. Dermatol. Case Rep., 2013, 2, 31 – 41.
15. Mehreagan, D.A. and Mehregan, A. H. Histopathology of cutaneous leishmaniasis,The gulf J. Dermatol. Venereol., 1997, l4(2), 1-9.
16. Sharaquie, K.E. AlTalib, K.K. and Chu, A.C. Intralesional therapy of cutaneosleishmaniasis with sodium stibogluconate antimony, Br. J. Dermatol., 1988, 119(1), 53-57.
17. Al-Jeboori, T.I. and Evans, D.A. Leishmania species in Iraq: Electrophoresis and isoenzyme patterns, Trans. Roy. Soc. Trop. Med. Hyg., 1980, 74, 178-179.
18. Arroub H.; Alaoui A. Lemrani , M and Habbari K. Cutaneous Leishmaniasis in FoumJamâa (Azilal, Morocco):Micro-Environmental and Socio-Economical Risk Factors J. Agric. Soc. Sci., 2012, 8,10–1
19. Bailey, M.S. and Diana N.J. Cutaneous leishmaniasis. Clin.Dermatol.,2007, 25, 203- 211.
20. Rastogi, V. and Nirwan, P.S. Cutaneous leishmaniasis: an emerging infection in a non-endemic area and a brief update. Indian J. Med Microbiol., 2007, 25,272-275.

21. Stewart, C.C. and Brieger, W.R. Community views on cutaneous leishmaniasis in Istalif, Afghanistan: implications for treatment and prevention. Int Quart Commun Health Education, 2009, 29, 123-142.
22. Fellah, H.; Rhajoui, M.; Ouahabi, S.; Belghiti, D. and Lyagoubi, M. Occurrence of Human Cutaneous Leishmaniasis in Zouagha my Yacoub Province (Morocco). Int. J. Agri. Biol., 2007, 9 (1), 197-198.
23. Gurel, M.S.; Ulukanligil, M. and Ozbilge, H. Cutaneous leishmaniasis in Sanliurfa: epidemiologic and clinical features of the last four years (1997-2000). Int. J.Dermatol.,2002, 41(1), 32-7.
24. Aytekin, S.; Ertem, M.; Yağdıran, O.andAytekin, N. Clinico epidemiologic study of cutaneous leishmaniasis in Diyarbakir Turkey. Dermatol Online J.,2006, 12(3), 14.
25. Yaghoobi-Ershadi, M.R.; Akhavan, A.A.; Zahraei-Ramazani, A.V. Abai, M.R.; Ebrahimi, B.; Vafaei-Nezhad, R.; Hanafi-Bojd, A.A. and Jafari, R. Epidemiological study in a new focus of cutaneous leishmaniasis in the Islamic Republic of Iran. East Med. Health J., 2003, 9(4), 816-26.
26. Al-Samarai,A.M. and AlObaidi,H.S.Cutaneous leishmaniasis in Iraq ,J. Infect developing countries,2009, 3(2),123-129.
27. Khalid,A.;Ahmed,S.A.;Haque,A.U. and Mukhtar, R. Diagnostic microscopic features of cutaneous leishmaniasis other than *Leishmaniatropica* bodies,International J. Pathol.,2012, 10 (1),21-25.
28. Magill,A.J. Epidemiology of the leishmaniasis.Dermatoepidemology,1995, 13,505- 523.
29. Gumurdulu,D.; Ergin,M.; Tuncer,I.;Uzun,S. and Memisoglu,H. Histopathological and clinical evalution of the cutaneous leishmaniasis in southern Anatolia, Turkey, Aegean. Pathol. J. 2004, 1(1), 57-61.
30. Sharquie, K.E. and Hameed, A.F. Panniculitis is an important feature of cutaneous leishmaniasis pathology, Case reports in Dermatological Med.,2012, 612434.
31. Andrade-Narvaez, F.J.; Medina-peralta,S.;Vargas-Gonzales,A.; Canto-lara,S.B. and Estrada-Parra,S. The histopathology of cutaneous leishmaniasis due to leishmaniamexicana in the yucatan peninsula, Mexico, Rev. Inst. Med. Trop. S. Paulo,2005, 47(4), 191-194.
32. Moravvej, H.; Barzegar, M.; Nasiri,S.;Abolhasani, E. and Mohebali, M. Cutaneous leishmaniasis with unusual clinical and histological presentation: Report of four cases, ActaMedicaIranica, 2013,51(4), 274-278.

*****