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EVALUATION OF CUTANEOUS LEISHMANIA CASES AND DIAGNOSTIC METHODS IN BATMAN

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Abstract

Objective: The diagnostic method used in cutaneous leishmaniasis (CL) often depends not on the accuracy of the diagnosis but on the existing infrastructure and resources of the diagnostic facility. It is important to apply a practical and sensitive method in regions where diagnostic possibilities are limited. This study aims to examine the cases diagnosed with CL and the diagnosis method in Batman between July 2021 and July 2023.

Methods: Totally 17 patients were referred to the Microbiology Laboratory with a prediagnosis of CL. Demographic data of the patients were obtained from the Hospital Information Management System. The fine needle aspiration method was preferred as the diagnostic method in 2021, the samples were taken by partial removal of the suspected crust of scar/ulcer in 2022 and 2023.

Result: With the suspicion of Leishmaniasis lesion, 5 patients in 2021, 8 patients in 2022 and 4 patients in 2023 were referred to our laboratory. Samples were taken with the fine needle aspiration method in 2021 and *Leishmania sp.* amastigotes were detected in one of five patients. In the samples taken in 2022 and 2023, the sample was taken by partial removal of the crust. *Leishmania sp.* amastigotes were detected in 6 of 8 suspected patients in 2022 and in 3 of 4 suspected patients in 2023.

Conclusion: In this study, 10 CLs were diagnosed and it was observed that taking samples by removing the wound/ulcer crust made it easier to detect the agent. All the cases were local and were thought to be independent of Syrian migration.

Keywords: *Cutaneous leishmaniasis,diagnostic method, sand flies, parasite.*

Introduction

Leishmaniasis infection can occur in humans in three different clinical forms: visceral leishmaniasis (VL), which is a life-threatening condition, cutaneous leishmaniasis (CL), which is usually a more benign form causing ulcerated skin lesions and mucocutaneous leishmaniasis (MCL) which can lead to partial or total destruction of mucous membranes. Four *Leishmania* species are endemic in countries bordering the Mediterranean and Black Seas. *L. donovani complex species* (*L. infantum* and *L. donovani*) cause VL and *L. major* and *L. Tropica* cause CL.[1,2]

Leishmania sp. parasite is transmitted by the bite of sand flies belonging to the genus *Phlebotomus* and *Lutzomyia*. The parasite's reservoirs consist of wild or semi-domesticated animals, usually rodents or dogs. The parasite located in the gut of the vector is a unicellular flagellated protozoan containing intracellular organisms and a kinetoplast.[3]

Leishmaniasis infection can occur in humans in three different clinical forms: visceral leishmaniasis (VL), which is a life-threatening condition, cutaneous leishmaniasis (CL), which is usually a more benign form causing ulcerated skin lesions and mucocutaneous leishmaniasis (MCL) which can lead to partial or total destruction of mucous membranes. Four *Leishmania* species are endemic in countries bordering the Mediterranean and Black Seas. *L. donovani complex species* (*L. infantum* and *L. donovani*) cause VL and *L. major* and *L. Tropica* cause CL.[1,2]

Cutaneous leishmaniasis is endemic in almost 100 countries, annually two million new cases occur and CL is the most common leishmanial manifestation.[4] Turkiye is an endemic country for *Leishmania* infection. More than 90% of Leishmaniasis cases are from Afghanistan, Algeria, Brazil, Iran, Pakistan, Peru, Saudi Arabia, and Syria.[5] CL outbreaks occurred due to healthcare disruption, wars, conflicts such as in Syria. Outbreaks occurred not only in war zones, but also in countries such as Turkiye, Jordan, and Lebanon, where refugees migrated from those regions.[6]

The diagnostic method used in *Leishmania* infection often depends not on the accuracy of the diagnosis but on the existing infrastructure and resources of the diagnostic facility. It is important to apply a practical and sensitive method in regions where diagnostic possibilities are limited. The aim of this study is to examine the cases diagnosed with CL and the diagnosis method in Batman between July 2021 and July 2023.

Methods

Patients with a prediagnosis of CL referred to the Microbiology Laboratory by the Dermatology Clinic were investigated. Between July 2021 and July 2023, 17 patients were referred to the Microbiology Laboratory with a prediagnosis of CL. All of the cases in this study were local cases, none of the patient have history of traveling abroad. Demographic data of the patients were obtained from the Hospital Information Management System. The fine needle aspiration method was preferred as the diagnostic method in 2021, the samples were taken by partial removal of the suspected crust of scar/ulcer in 2022 and 2023. Fine-needle aspiration method was applied in the form of disinfecting the wound area with 70% ethanol, injecting 0.5 ml of saline from the periphery of the wound into the ulcer/wound floor and collecting the fluid. In the other method, after 70% ethanol disinfection of the skin, partial removal of the wound/ulcer crust (2-3 mm) and applying a sterile swab from the serous discharge underneath. The samples were fixed with 100% methanol and stained with Giemsa dye before being examined with a light microscope, under the x100 objective with oil immersion. Diagnostic criterion for CL was the presence of Leishmania amastigotes in the samples.

Result

With the suspicion of Leishmaniasis lesion, five patients in 2021, 8 patients in 2022 and four patients in 2023 were referred to our laboratory. Samples were taken with the fine needle aspiration method in 2021 and *Leishmania sp.* amastigotes were detected in one of 5 patients. In the samples taken in 2022 and 2023, the sample was taken by partial removal of the crust. *Leishmania sp*. amastigotes were detected in 6 of 8 suspected patients in 2022 and in three of four suspected patients in 2023. A total of 10 patients were diagnosed with CL by direct microscopic diagnosis. Of the patients, 5 (50%) were female and 5 were male (50%). The ages of the patients ranged from 0 to 47, with a mean age of 23.4 years. All of the patients live in Batman and have no history of traveling abroad. Of the cases, 6 (60%) applied to the dermatology clinics in the spring season and 4 (40%) in the winter season. The lesions were on the face in 2 patients, on the neck in one, and on the hands and arms in the others. The lesions were multiple in three patients. Examples of lesions and microscopic images of *Leishmania sp.* amastigotes of the patients are given in Figure 1.

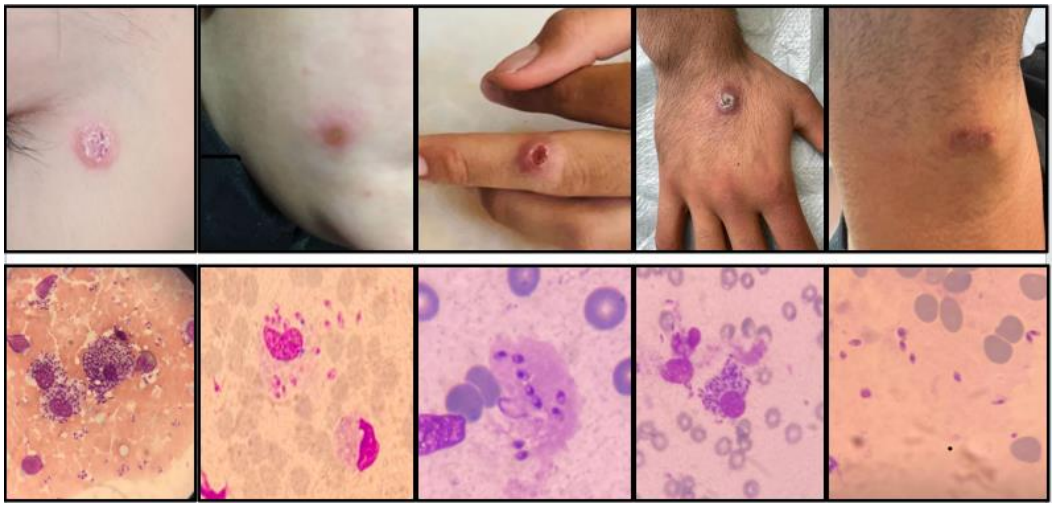

Figure 1: Examples of lesions and microscopic images of Leishmania amastigotes of the patients

Discussion

CL is endemic in the Southeastern Anatolia region of Turkiye, one of these provinces is Batman.[7] It has been reported that CL is more common in children and women in regions where the disease is endemic.[8] It is known that CL is more common in the younger age group. In the study of Korkmaz et al. in 2015 with 635 CL patients, it was found that CL was most common in the 10-19 age group.[9] In this study, the number of men and women diagnosed with CL was equal. Of the CL cases 3 were children (11 months, 1 and 9 years old), the others were adults. The mean age was 23.4 years, and similar to the studies, the cases were in the younger age group. The months in which case reports are made vary in studies.[8-10] In this study, it was observed that most of the cases (60%) detected within a 2-year periods applied in the spring season.

In the study conducted by Korkmaz et al with 635 CL cases in Gaziantep province, 89.4% of the cases were Syrian.[9] In the study of Yazısız et al. in 2020, in which they examined 195 cases, it was shown that 78.6% of the cases were composed of immigrants from Syria.[11] Due to the frequent occurrence of the disease in refugees who migrated to Turkiye due to the civil war in Syria, the disease has gained an important dimension especially in Southeastern Anatolian provinces.[12] However, it has been observed that all of the cases in this study were local cases, and it was thought that the vector and the agent were present in Batman and were not affected by migration. It is known that in most of the CL cases, the lesions are mostly observed on the face and upper extremities.[12,13] It is similarly located in the face and upper extremities in the cases in this study.

Although there are concerns about its sensitivity, direct parasitological diagnosis is still considered the gold standard for the diagnosis of leishmaniasis due to its high specificity.[14] Direct parasitological diagnosis can be made by histopathological analysis of formalin-embedded tissue or in vitro parasite culture from specimens from suspicious lesions. *Leishmania* amastigotes can be detected directly in lesional smears of biopsies by staining with the Giemsa method. Amastigotes are defined as round or oval structures 2–4 µm in diameter with typical nuclei and kinetoplasts.[15] In addition, real-time PCR (qPCR)-based approaches in the diagnosis of CL have become increasingly popular in recent years not only for the detection and quantification of *Leishmania* species, but also for species identification.[16] However, molecular diagnosis is not an easily accessible method in every region of Turkiye due to reasons such as cost, lack of equipment and lack of experienced personnel. Serological tests are not preferred in the diagnosis of *Leishmania* due to weak humoral immune response and therefore low sensitivity.[15] Cultivation of *Leishmania sp*. takes place in tubes containing Novy-Mac-Neal-Nicolle medium from suspected lesions. Parasite culture is difficult, requires significant technical expertise, is prone to contamination and is time consuming. The sensitivity of culture tends to be low and highly variable.[17]

In countries where the disease is not common, the availability of laboratory facilities provides adequate and effective follow-up of the disease. However, in developing countries with large numbers of patients in rural areas, simple diagnostic tools are required for field use.[18] In our laboratory, the diagnosis of CL is made by staining and evaluating the samples taken from the lesion area microscopically with Giemsa. As a result of the evaluation, although the fine needle aspiration method is more comfortable for the patient, taking a direct sample from the serous discharge by partially removing the crust of ulcer/scar makes it easier to detect the agent. One of the disadvantages of the removing crust method is the risk of contamination by opening the wound to external factors and the possibility of secondary infections. In the follow-up of the cases, no secondary infections were found in the lesion. Due to the small number of cases, the sensitivity of the methods and possible complications could not be compared statistically.

As a result of this study, 10 CLs were diagnosed in the laboratory between July 2021 and July 2023, and it was seen that taking samples by removing the wound/ulcer crust made it easier to detect the agent. All the cases were local cases, and the cases detected in Batman were thought to be independent of Syrian migration.

Conflict of Interest

The authors have no conflicts of interest to disclose.

Ethical Approval

Ethics committee approval was obtained from Batman Training and Research Hospital (2023/360).

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References

1. Bennis I, Verdonck K, El Khalfaoui N, et al. Accuracy of a Rapid Diagnostic Test Based on Antigen Detection for the Diagnosis of Cutaneous Leishmaniasis in Patients with Suggestive Skin Lesions in Morocco. *Am J Trop Med Hyg*. 2018;99(3):716-722. doi:10.4269/ajtmh.18-0066.
2. Roberts T, Keddie SH, Rattanavong S, et al. Accuracy of the direct agglutination test for diagnosis of visceral leishmaniasis: a systematic review and meta-analysis. *BMC Infect Dis*. 2023;23(1):782. doi:10.1186/s12879-023-08772-1.
3. Mokni M. [Cutaneous leishmaniasis]. *Ann Dermatol Venereol*. 2019;146(3):232-246. doi:10.1016/j.annder.2019.02.002.
4. de Vries HJC, Schallig HD. Cutaneous Leishmaniasis: A 2022 Updated Narrative Review into Diagnosis and Management Developments. *Am J Clin Dermatol*. 2022;23(6):823-840. doi:10.1007/s40257-022-00726-8.
5. Burza S, Croft SL, Boelaert M. Leishmaniasis. *Lancet* (London, England). 2018;392(10151):951-970. doi:10.1016/S0140-6736(18)31204-2.
6. Ozaras R, Leblebicioglu H, Sunbul M, et al. The Syrian conflict and infectious diseases. *Expert Rev Anti Infect Ther*. 2016;14(6):547-555. doi:10.1080/14787210.2016.1177457.
7. Ok UZ, Balcioğlu IC, Taylan Ozkan A, Ozensoy S, Ozbel Y. Leishmaniasis in Turkey. *Acta Trop*. 2002;84(1):43-48. doi:10.1016/s0001-706x(02)00134-1.
8. Çulha G, Akçali C. Hatay ve Çevresinde Saptanan Kutanöz Leishmaniasis Olguları Detection of Cutaneous Leishmaniasis Cases in Hatay and Surrounding Areas. *Türkiye Parazitoloji Derg*. 2006;30(4):268-271.
9. Korkmaz S, Özgöztaşı O, Kayıran N. The Assesment of Cutaneous Leishmaniasis Patients Admiting to Gaziantep University of Medicine Faculty Leishmaniasis Diagnosis and Treatment Center. *Türkiye Parazitoloji Derg.* 2015;39(1):13-16. http://search/yayin/detay/197776.
10. Gurel MS, Ulukanligil M, Ozbilge H. Cutaneous leishmaniasis in Sanliurfa: epidemiologic and clinical features of the last four years (1997-2000). *Int J Dermatol*. 2002;41(1):32-37. doi:10.1046/j.0011-9059.2001.01396.x.
11. Yazısız H, Çekin Y, Aydın Tığlı G, Koçlar FG, Gür N. Retrospective Evaluation of Cutaneous Leishmaniasis Results: Data from a Tertiary Hospital in Antalya. *Akdeniz Med J*. 2020;6(3):506-510.

12. Aytekin S, Ertem M, Yağdiran O, Aytekin N. Clinico-epidemiologic study of cutaneous leishmaniasis in Diyarbakir Turkey. *Dermatol Online J*. 2006;12(3):14.
13. Yemisen M, Ulas Y, Celik H, Aksoy N. Epidemiological and clinical characteristics of 7172 patients with cutaneous leishmaniasis in Sanliurfa, between 2001 and 2008. *Int J Dermatol*. 2012;51(3):300-304. doi:10.1111/j.1365-4632.2011.05059.x.
14. de Vries HJC, Reedijk SH, Schallig HDFH. Cutaneous leishmaniasis: recent developments in diagnosis and management. *Am J Clin Dermatol*. 2015;16(2):99-109. doi:10.1007/s40257-015-0114-z.
15. Goto H, Lindoso JAL. Current diagnosis and treatment of cutaneous and mucocutaneous leishmaniasis. *Expert Rev Anti Infect Ther*. 2010;8(4):419-433. doi:10.1586/eri.10.19.
16. Galluzzi L, Ceccarelli M, Diotallevi A, Menotta M, Magnani M. Real-time PCR applications for diagnosis of leishmaniasis. *Parasit Vectors*. 2018;11(1):273. doi:10.1186/s13071-018-2859-8.
17. Boggild AK, Miranda-Verastegui C, Espinosa D, et al. Optimization of microculture and evaluation of miniculture for the isolation of Leishmania parasites from cutaneous lesions in Peru. *Am J Trop Med Hyg*. 2008;79(6):847-852.
18. Santarém N, Cordeiro da Silva A. Diagnosis of visceral leishmaniasis. *In: Communicating Current Research and Educational Topics and Trends in Applied Microbiology*. ; 2007:839-846.