Vascular patterns in cutaneous leishmaniasis: A videodermatoscopic study

Sir,

Leishmaniasis includes a spectrum of mammalian diseases caused by a parasite protozoan classified as Leishmania species.[1] Primary skin infection i.e. cutaneous leishmaniasis sometimes clears up without treatment, with the development of an acquired immunity through cellular and humoral responses. However, the infection can spread causing secondary lesions in the skin that represent a reaction to the local parasite or to its antigenic products, or in the mucosa and in visceral organs.[2]

Zoonotic *Leishmania infantum* is transmitted in both the Eastern and the Western hemispheres. In Europe, there are risks of emergence or re-emergence of leishmaniasis which include the spread of visceral and cutaneous leishmaniasis caused by *L. infantum* from the Mediterranean region of Europe, increased number of immunosuppressed people, and high prevalence of asymptomatic human carriers of *L. infantum* in southern Europe.

Clinical examination of suspected cases, parasitological diagnosis and immunodiagnosis are the routine methods available for the diagnosis of leishmaniasis. Molecular tests have been used to identify *Leishmania* infections, but there has been no international standardization. Monoclonal antibodies have long been available for the identification of *Leishmania* species but they are not widely used. Some consider multi-locus enzyme electrophoresis (MLEE) which is used to identify *Leishmania* species and strains, as the gold standard.[3] This method requires axenic culture in which one strain can overgrow others in mixed infections. Polymerase chain reaction of the internal transcribed spacer of the multi-copy nuclear ribosomal genes is often used. A set of carefully chosen criteria must accompany polymerase chain reaction-based diagnosis, especially for immunocompromised patients.

Videodermatoscopy is a non-invasive technique, which has also been employed for the evaluation of inflammatory skin disorders and infectious disease,[4,5] that provides additional information useful to address the diagnosis by evaluating the superficial vascular pattern. We considered interesting to describe the dermatoscopic features of cutaneous leishmaniasis, compared with data obtained from the microscopic identification of parasites and with data obtained from polymerase chain reaction method for diagnosis or identification of *Leishmania* from human cutaneous samples.

The study was performed in the dermatology department of the University of Palermo, Italy.

Samples were taken from 21 consecutive patients (9 women and 12 men) with 23 cutaneous leishmaniasis lesions. Informed consent was obtained. Informed consent was premised on the participants' understanding the scope of the research and the associated risks and benefits. All patients were infected in Sicily. In all cases, the diagnosis of leishmaniasis

was confirmed by the demonstration of amastigotes in Giemsa-stained smears. Polymerase chain reaction was also performed as confirmation test.[6] DNA from each slide was extracted separately and the real time polymerase chain reaction test was targeted on the constant region within the mini circle kinetoplast DNA.[7] The tests were conduct by using Abi Prism 7700 Sequence Detector (Applied Biosystem), using TaqMan Master Mix (Applied Biosystem). A positive control containing genomic *L. infantum* DNA and a negative control without DNA were included in all the assays. The parasitic DNA charge was determined in each examined sample by comparison of the data with a specific standard curve based on the number of Leishmania per milliliter of extracted volume. Specimens were considered confirmed positives (C-Pos) when stained tissue smears were positive for parasites or at least two polymerase chain reaction assays were positive for leishmanial DNA.

Dermoscopic photographs of each lesion were taken using the EasyScan® system; videodermatoscopy examination was performed using two different magnifications at ×30 and ×150. All the dermoscopic images were acquired before therapy.

The age of the study subjects varied from 2 to 75 years with a mean age of 43.8 years. Lesions were located on the face (4), on the arms (8), on legs (9) and on trunk (2). The videodermatoscopy study of the 23 lesions demonstrated, similar to previous studies by Llambrich *et al.*,[8] Yücel A *et al.*[9] and Taheri AR *et al.*,[10] the existence of the following dermoscopic features: generalized erythema in all lesions (100%), vascular structures in all lesions (100%), central erosion/ulceration in 14 cases (60.8%), thrombotic vessels in 9 cases (39.1%), 'yellow tears' in 9 cases (39.1%), 'white starburst-like pattern' in 5 cases (21.7%), hyperkeratosis in 5 cases (21.7%), and erosion/ulceration with hyperkeratosis in 3 cases (13.0%) [Table 1].

Vascular structures that we identified were in order of frequency: linear irregular vessels, being present in 22 (95.6%) of 23 cases, hairpin vessels in 14 (60.8%) cases, comma-shaped vessels, which were present in 14 (60.8%) cases, dotted vessels in 9 (39.1%) cases, arborizing telangiectasia in 3 (13.0%) cases, glomerular-like vessels in 3 (13.0%) cases, corkscrew vessels in 1 (4.3%) case [Table 1]. Two or more different types of vascular structures were present in all lesions (100%); only two vascular structures were present in 9 (39.1%) cases and three or more vascular structures were present in 14 (60.8%) cases.

Table 1: Dermoscopic features of cutaneous leishmaniasis lesions

Dermoscopic features	Initial cutaneous leishmaniasis (%)	Advanced cutaneous leishmaniasis (%)	Cutaneous leishmaniasis, present study (%)
Generalized erythema	60.8	39.1	100
Erosion/central ulceration	39.1	21.7	60.8
Thrombotic vessels	30.4	8.7	39.1
Yellow tears	21.7	17.3	39.1
White starburst	4.3	17.3	21.7
Hyperkeratosis	13.0	8.7	21.7
Hyperkeratosis + erosion/central ulceration	0	13.0	13.0
Vascular patterns	60.8	39.1	100
Dilated/ telangectasic vessels	100	39.1	100
Linear irregular vessels	56.5	39.1	95.6
Hairpin vessels	34.7	26.0	60.8
Comma-shaped vessels	39.1	21.7	60.8
Dotted vessels	21.7	17.3	39.1
Glomerular-like vessels	8.7	4.3	13.0
Arborizing telangectasia	8.7	4.3	13.0
Corkscrew vessels	4.3	0	4.3

Initial lesions (<6 months of duration; n = 14 lesions) showed: Erosion (64.2%), thrombotic vessel (50.0%), yellow tears (35.7%), white starburst-like pattern (7.1%), hyperkeratosis (21.4%)). In initial lesions, the vascular structures were linear irregular vessels (92.8%), comma-shaped vessels (64.2%), hairpin vessels (57.1%), dotted vessels (35.7%), arborizing telangiectasia (14.2%), glomerular-like vessels (14.2%), and corkscrew vessels (7.1%).

Advanced lesions (>6 months of duration; n = 9 lesions) showed: erosion (55.5%), thrombotic vessel (22.2%), yellow tears (44.4%), white starburst like pattern (44.4%), hyperkeratosis (22.2%), both erosion and hyperkeratosis together (33.3%). In advanced lesions, the vascular structures were: linear irregular vessels (100%), hairpin vessels (66.6%), comma-shaped vessels (55.5%), dotted vessels (44.4%),

arborizing telangiectasia (11.1%), glomerular-like vessels (11.1%), but no corkscrew vessels.

Dilated and telangectatic vessels and polymorphous vessels[11] were present in all cases both in initial and advanced lesions. These had higher prevalence rates in those cases that presented higher polymerase chain reaction values, especially in advanced (≥6 months) lesions (r = 0.8) [Figures 1 and 2].

The term "leishmaniasis" defines a group of vector-borne diseases caused by species of the genus *Leishmania*. Human infection may display a spectrum of clinical manifestations from cutaneous involvement to generalized systemic visceral disease with fatal outcome. Sicily is one of the major islands of the Mediterranean Basin and it is considered a hypo-endemic area for showing an incidence of approximately 0.20 per 1000 inhabitants.

Surveillance data has indicated that the global number of cutaneous leishmaniasis cases in the Mediterranean Basin has increased during the past decade. Originally it was a rural problem, but now it is becoming common in urban areas. Such an increase has also been observed in Sicily, and can be explained, in part, by the presence of sand flies in this region during a wide period of the year. This is the result of the climatic variation in recent years (increasing temperature and humidity). This permits the perpetuation of the sand fly life cycle.

The clinical features of cutaneous leishmaniasis may vary in terms of type and extension, ranging from single, chronic ulcerative lesions to disseminated nodular lesions displaying diverse intensities of dermal inflammatory infiltrate.[12] Lesions exhibit granulocytes and mononuclear cell infiltration in the early phases changing to a pattern with numerous lymphocytes and macrophages later in the lesion.

Therefore, different chemokines may affect the predominance of cell infiltration in distinct clinical manifestations. Chemokines facilitate leukocyte migration and positioning as well as other processes such as angiogenesis and leukocyte degranulation.[13] Some chemokines also stimulate angiogenesis or angiostasis. The biological relevance of angiogenic or angiostatic properties of chemokines could relate to inflammatory responses where angiogenesis is an important requirement.

Local inflammation during cutaneous leishmaniasis is accompanied by accumulation of CD11b + cells at the site of the infection. Leishmania species are obligate intracellular parasites of cells of the macrophage-dendritic cell lineage. Functional correlations between the plasticity and angiogenic properties of specific myelomonocytic populations or macrophage precursors have been detailed in recent reports.[14-16] In the later phases of the infection, macrophages can eliminate the parasites after activation by interferon gamma producing T helper type 1 (Th1) cells.[17] Carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM1), expressed on CD11b^{high} cells is engaged in homotypic and heterotypic adhesion processes during cellular growth and proliferation or innate and inflammatory immune responses.[18-20] CEACAM is essential for angiogenesis in inflammation.

Videodermatoscopy is a non-invasive technique that has also been employed for the evaluation of inflammatory skin disorders and infectious disease, which provides additional information useful to address the diagnosis by evaluating the superficial vascular pattern. In our study, the most common dermoscopic pattern

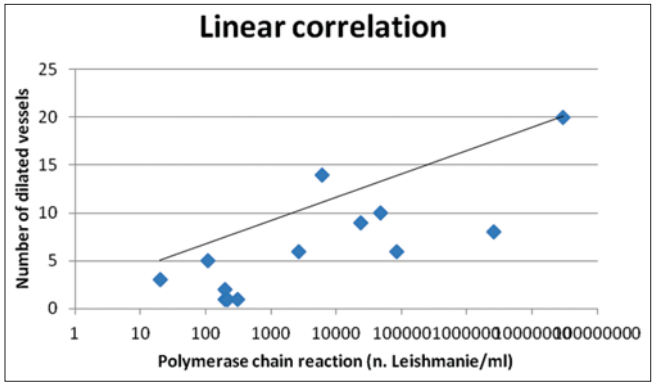

Figure 1: Correlation between initial lesions and polymerase chain reaction (n. Leishmanie/ml), Linear Correlation: 0.5

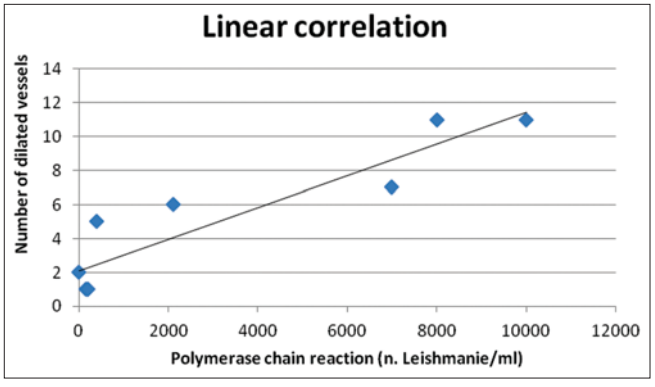

Figure 2: correlation between advanced lesions and polymerase chain reaction (n. Leishmanie/ml), Linear Correlation: 0.8

of initial lesions of cutaneous leishmaniasis were: erythema and erosion [Figure 3a and b]; in advanced lesions: erythema, erosion, yellow tears, and white starburst [Figure 4a and b]. In two cases, we identified only vascular patterns [Figure 5a and c].

In this study, we compared vascular pattern to polymerase chain reaction value. Number of dilated and telangiectatic vessels were more frequent in those cases that present higher polymerase chain reaction values, especially in advanced (≥6 month) lesions (r = 0.8). We speculate that this higher value of polymerase chain reaction could be correlated to an increased angiogenesis, which results in a higher frequency of dilated and telangectasic vessels that can be highlighted by using videodermatoscopy.

We propose that the use of videodermatoscopy should be encouraged, especially for the speed and specificity of the information that can be obtained; it could help to improve the accuracy of clinical diagnosis of the disease. In addition, being non-invasive, the technique is easy for physicians to undertake and may reduce, in certain situations, the need for invasive diagnostic measures.

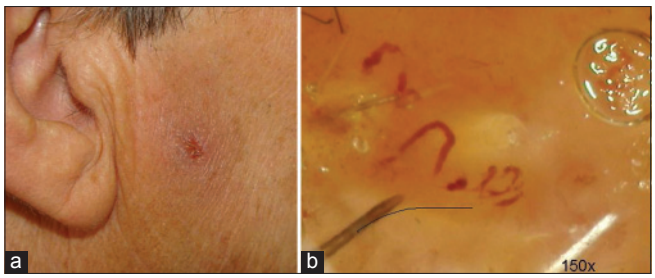

Figure 3: (a) Cutaneous lesion showing erythema and erosion. (b) Numerous dilated vessels in the erythematous area

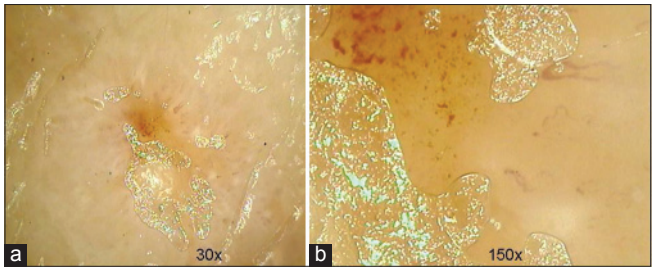

Figure 4: (a) Erythema, erosion, yellow tears, and thrombotic vessel in a lesion present for 10 months before videodermoscopy ×30. Quantitative PCR (n. Leishmanie/ml): 8000 leish/ml. (b) Dilated and thrombotic vessel observed with videodermoscopy ×150

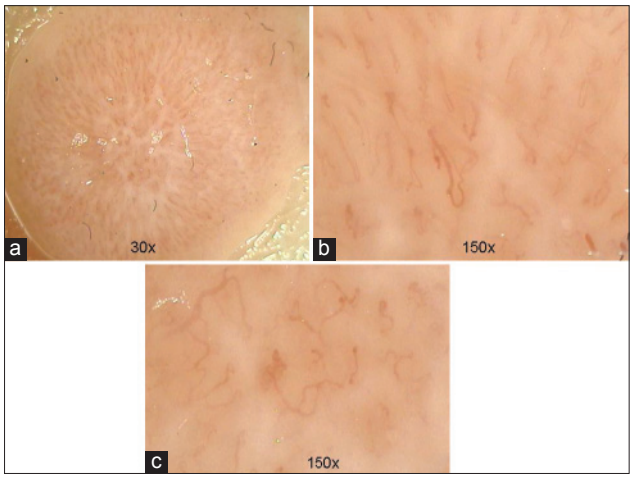

Figure 5: (a) Only vascular pattern in a nodular lesion, that has been present for 7 months. Quantitative PCR (n. Leishmanie/ml): 48000 leishmanie/ml. (b) Hairpin vessels at the periphery of the cutaneous lesion. (c) Arborizing telangectasia, linear irregular vessels, comma-shaped vessels

Filomena Caltagirone, Giuseppe Pistone, Mario Arico, Fabrizio Vitale[1], Daniele Sorbello, Daniele Rizzo, Maria Rita Bongiorno

Department of Dermatology, University of Palermo, [1]Department of C.Re.Na.L, Istituto Zooprofilattico Sperimentale Della Sicilia "A. Mirri", Palermo, Italy

Address for correspondence: Prof. Maria Rita Bongiorno, Department of Dermatology, University of Palermo, Palermo, Italy.
E-mail: mariarita.bongiorno@unipa.it

REFERENCES

1. Guenin-Macé L, Oldenburg R, Chrétien F, Demangel C. Pathogenesis of skin ulcers: Lessons from the Mycobacterium ulcerans and Leishmania spp. pathogens. Cell Mol Life Sci 2014;71:2443-50.
2. Singh OP, Hasker E, Sacks D, Boelaert M, Sundar S. Asymptomatic leishmania infection: A new challenge for leishmania control. Clin Infect Dis 2014;58:1424-9.
3. Van der Auwera G, Ravel C, Verweij JJ, Bart A, Schönian G, Felger I. Evaluation of four single-locus markers for Leishmania species discrimination by sequencing. J Clin Microbiol 2014;52:1098-104.
4. Zalaudek I, Argenziano G, Di Stefani A, Ferrara G, Marghoob AA, Hofmann-Wellenhof R, *et al*. Dermoscopy in general dermatology. Dermatology 2006;212:7-18.
5. Zalaudek I, Giacomel J, Cabo H, Di Stefani A, Ferrara G, Hofmann-Wellenhof R, *et al*. Entodermoscopy: A new tool for diagnosing skin infections and infestations. Dermatology 2008;216:14-23.
6. Lavergne RA, Iriart X, Martin-Blondel G, Chauvin P, Menard S, Fillaux J, *et al*. Contribution of molecular diagnosis to the management of cutaneous leishmaniasis in travellers. Clin Microbiol Infect 2014;20:0528-30.
7. Reale S, Maxia L, Vitale F, Glorioso NS, Caracappa S, Vesco G. Detection of Leishmania infantum in dogs by PCR with lymph node aspirates and blood. J Clin Microbiol 1999;37:2931-5.
8. Llambrich A, Zaballos P, Terrasa F, Torne I, Puig S, Malvehy J. Dermoscopy of cutaneous leishmaniasis. Br J Dermatol 2009;160:756-61.
9. Yücel A, Günaşti S, Denli Y, Uzun S. Cutaneous leishmaniasis: New dermoscopic findings. Int J Dermatol 2013;52:831-7.
10. Taheri AR, Pishgooei N, Maleki M, Goyonlo VM, Kiafar B, Banihashemi M, *et al*. Dermoscopic features of cutaneous

leishmaniasis. Int J Dermatol 2013;52:1361-6.
11. Zalaudek I, Kreusch J, Giacomel J, Ferrara G, Catricalà C, Argenziano G, *et al*. How to diagnose nonpigmented skin tumors: A review of vascular structures seen with dermoscopy: Part II. Nonmelanocytic skin tumors. J Am Acad Dermatol 2010;63:377-86.
12. Bongiorno MR, Pistone G, Aricò M. Unusual clinical variants of cutaneous leishmaniasis in Sicily. Int J Dermatol 2009;48:286-9.
13. Mackay CR. Chemokines: Immunology's high impact factors. Nat Immunol 2001;2:95-101.
14. Maruyama K, Ii M, Cursiefen C, Jackson DG, Keino H, Tomita M, *et al*. Inflammation-induced lymphangiogenesis in the cornea arises from CD11b-positive macrophages. J Clin Invest 2005;115:2363-72.
15. Kerjaschki D. The crucial role of macrophages in lymphangiogenesis. J Clin Invest 2005;115:2316-9.
16. Seandel M, Butler J, Lyden D, Rafii S. A catalytic role for proangiogenic marrow-derived cells in tumor neovascularization. Cancer Cell 2008;13:181-3.
17. Belkaid Y, Piccirillo CA, Mendez S, Shevach EM, Sacks DL. CD4 + CD25 + regulatory T cells control Leishmania major persistence and immunity. Nature 2002;420:502-7.
18. Kuespert K, Pils S, Hauck CR. CEACAMs: Their role in physiology and pathophysiology. Curr Opin Cell Biol 2006;18:565-71.
19. Horst AK, Ito WD, Dabelstein J, Schumacher U, Sander H, Turbide C, *et al*. Carcinoembryonic antigen-related cell adhesion molecule 1 modulates vascular remodeling *in vitro* and *in vivo*. J Clin Invest 2006;116:1596-605.
20. Mylonas J, Nair MG, Prieto-Lafuente L, Paape D, Allen JE. Alternatively activated macrophages elicited by helminth infection can be reprogrammed to enable microbial killing. J Immunol 2009;182:3084-94.

Access this article online

Quick Response Code:

Website: www.ijdvl.com

DOI: 10.4103/0378-6323.159939