

## Histopathological and clinical evaluation of the cutaneous leishmaniasis in Southern Anatolia, Turkey

Derya Gumurdulu<sup>1</sup>, Melek Ergin<sup>1</sup>, Ilhan Tuncer<sup>1</sup>, Soner Uzun<sup>2</sup>, Hamdi Memisoglu<sup>2</sup>  
*Departments of Pathology<sup>1</sup> and Dermatology<sup>2</sup>, Çukurova University Faculty of Medicine, Çukurova University Tropical Diseases Research Center<sup>2</sup>, Adana, Turkey*

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**Objective:** Cutaneous leishmaniasis (CL) continues to be an important world health problem. In Turkey, CL occurs most commonly in the region of Adana and its neighboring cities. Around 20 million people are at risk of infection in our country. In this study, we investigated the histopathological and clinical features of CL, as well as taking attention on its endemic importance.

**Study design:** Forty cases of which biopsy and/or smear examinations identified the leishmania parasites were evaluated clinically and histopathologically.

**Results:** The lesions of CL were found on facial regions in 19 cases and on extremities in 21 cases. Histopathologically, there was ulceration in 11 cases, epidermal atrophy in 9, acanthosis in 6, pseudoepitheliomatous hyperplasia in 8, granulomas in 18 cases.

**Conclusion:** While the number of parasites is concentrated in early lesions of CL, it may be difficult to find any parasites in older lesions. In a skin biopsy showing histiocytic infiltration and/or granulomatous reaction, CL should be included in the differential diagnosis. Currently, to recognize clinical and morphological features of the infection by clinicians and pathologists remains of great importance.

**Key words:** Cutaneous leishmaniasis, skin biopsy, smear, Turkey

### Introduction

Leishmaniasis is a disease caused by the leishmania parasites that occurs in three clinical forms: cutaneous, mucocutaneous and visceral leishmaniasis. It continues to be a major health problem at present. There are 350–400 million people at risk worldwide in around 82 countries with 400,000 new cases per year.<sup>1,2</sup> Turkey is an important geographical location for this disease because she is located at the point where three continents meet each other and surrounded by Caucasian, Middle Eastern, Mediterranean and Balkan nations, where different forms of leishmaniasis have been occurring for many years. *L. tropica* and *L. major* cause cutaneous leishmaniasis (CL) that has been a serious health problem in various parts of the country, foremost of that is the South Eastern Anatolian

region.<sup>1–4</sup> After the epidemic outburst of 1741 cases occurred in the South Eastern Anatolia region in 1983, disease became endemic in Cukurova region, especially in cities of Adana and Osmaniye.<sup>3–5</sup>

According to the report published by the Health Ministry of Turkish Republic, the majority of CL cases (98%) have occurred in the cities of Sanliurfa, Osmaniye, Adana, Hatay, Mersin and Kahramanmaras, where the disease is endemic. The estimated annual number of new cases in Turkey is around 5000.<sup>3</sup> At the Cukurova University Tropical Disease Research Center, an important source of data on CL in Turkey, the number of reported and confirmed cases is around 4500.<sup>3</sup> In this study, we evaluated the clinical and histopathological features of CL cases in our region aiming to take attention on this serious health problem.

## Materials and method

Forty cases with identified leishmania parasites in biopsy and/or smears were retrieved from the files of Pathology Department at Cukurova University, and a private pathology laboratory located in the City of Adana. The majority of cases were diagnosed at Department of Dermatology in Cukurova University, Faculty of Medicine, and at the Cukurova University Tropical Disease Research Center with recognizing typical macroscopic appearance and findings in Giemsa-stained smears of the lesions. In case when the parasite was not identified, two serial smears and a skin biopsy was performed. In 33 cases, paraffin-embedded tissue sections at 5µm thick were cut and stained with Hematoxylin-Eosin and Giemsa, whereas in the remaining 7 cases, touch imprint preparations from the lesions were stained with Giemsa for morphologic evaluation.

## Results

Of the cases, 29 (72.5%) were males, 11 (27.5%) females. Their ages ranged from 8 to 73 and the mean ( $\pm$  SD) age was  $41.3 \pm 16.6$  years. We observed that the lesions were of facial location in 19 (47.5%) cases including the cheeks in 9 cases, the forehead in 5, the earlobes in 1, the bottom lip in 1, the chin in 2 and above the eyebrow in 1, while in 21 cases (52.5%) they were found on the parts of extremities uncovered by clothing, such as the arms, the backs of the hands and the ankles. In 7 cases (17.5%), multiple lesions were observed. The size of the lesions ranged from 1 cm to 7 cm.

Clinically, the lesions started as small papule and within six months, they formed ulcers of 1–2 cm. These ulcers were painless with a necrotic base and an indurate margin and were frequently covered by a firmly adherent crust (Figure 1). These lesions healed as a scar within one year. The duration of the lesions ranged from 1 month to 36 months and the mean duration was 8.6 months. Among 40 cases, only 2 cases (5%) had chronic CL (duration of lesions was longer than 24 months).

Histopathologic studies revealed epidermal and dermal changes in 33 cases who had a biopsy. There were hyperkeratosis, parakeratosis, liquefaction degeneration of the basal cell layer and follicular



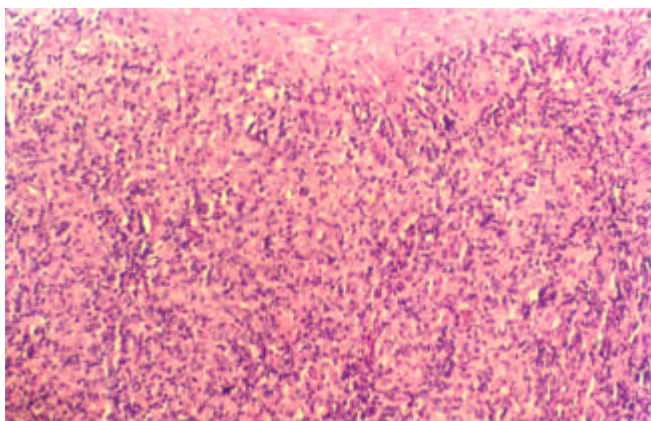
**Figure 1** An ulcerated nodule with a necrotic base and indurated margin.

plugging in the epidermis. An ulcer was present in 11 cases, epidermal atrophy in 9, acanthosis in 6, pseudoepitheliomatous hyperplasia in 8 and crust in 6. Epidermal findings are shown in Table 1. In all cases, a mononuclear dermal infiltration consisting of predominantly lymphocytes, histiocytes and plasma cells and occasional eosinophil leukocytes was seen (Figure 2).

**Table 1.** Histological epidermal findings of the CL cases

Epidermal findings	Number of cases (%)
Hyperkeratosis	19 (57.5%)
Parakeratosis	13 (39.3%)
Acanthosis	6 (18.1%)
Pseudoepitheliomatous hyperplasia	8 (24.2%)
Epidermal atrophy	9 (27.2%)
Follicular plugging	15 (45.4%)
Liquefaction degeneration of the basal cell layer	23 (69.6%)
Ulceration	11 (33.3%)
Crust	6 (18.1%)
Epidermal parasites	0 (0%)

In 18 cases (54.5%), granulomas without necrosis were found and half of them contained Langhans' type multinuclear giant cells. No free zone between epidermis and dermal infiltration was present. Dermal findings were summarized in Table 2. In all cases, Leishman-Donovan bodies, that are 2 to 4 µm, round

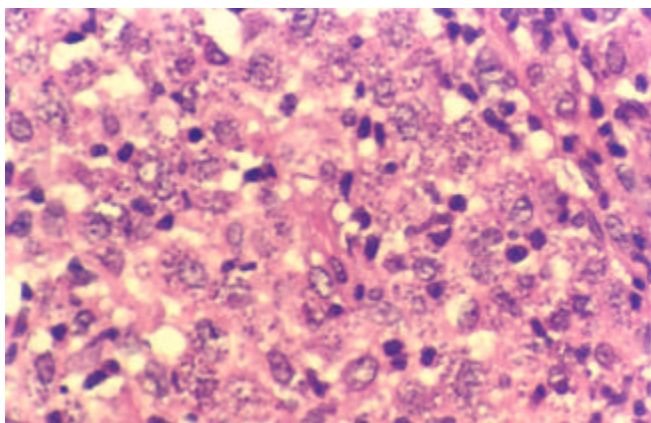


**Figure 2.** Dermal infiltration consisting of lymphocytes, histiocytes and eosinophil leukocytes (HE X 40).

or oval in shape were seen in large numbers within the macrophages and in the extracellular areas (Figures 3 and 4).

**Table 2.** Histological dermal findings of the CL cases

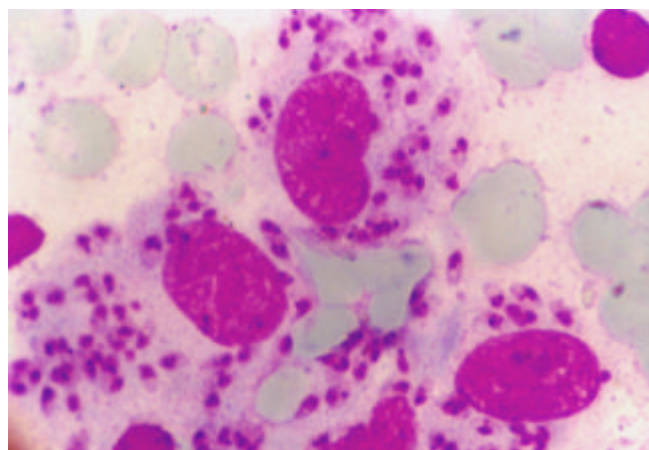
Dermal findings	Number of the cases (%)
Granuloma	18 (54.5%)
Giant cells (Langhans type)	9 (27.2%)
Cellular infiltration	
Histiocyte	33 (100%)
Lymphocyte	33 (100%)
Plasma cells	28 (84.8%)
Eosinophil leukocytes	14 (42.4%)



**Figure 3.** Leishman-Donovan bodies within the macrophages and extracellular (HE X 200)..

## Discussion

CL begins as a nodule or papule and becomes a chronic ulcer that is restricted to the skin and is the



**Figure 4.** The large numbers of Leishman-Donovan bodies within the macrophages in touch imprint preparation (Giemsa X 1000).

most common form of leishmaniasis. The most common agent in the Old World is *L. tropica* and *L. major*, in the New World *L. mexicana* and *L. braziliensis*.<sup>6</sup> In our country *L. tropica* is localized most often in southeastern and western regions, while *L. major* and its variants occur in widespread mountainous regions.<sup>7</sup>

The vector of CL has not been identified exactly in Turkey. In a study by Alptekin et al.<sup>5</sup>, *Phelebotomus sergenti* and *Phelebotomus papatasi* were two species of fly that were determined as the probable vectors during the last epidemic in Sanliurfa, a province in the southeast Turkey.

In recent years, non-endemic regions of our country such as city centers have also been found to have outbreaks of infection. One of the reasons that cause this situation is seasonal or permanent migrations of populations from endemic areas to the non-endemic areas. There have also been large agricultural and irrigation projects as well as dam and petroleum enterprises employing that increase the population of non-endemic origin in endemic areas. In addition, the use of insecticides inconsistently against the vector *phelobotomi* and the ecological changes brought about by large-scale dam and irrigation projects can also be considered. Even though CL is not a life threatening illness, it affects hundreds of people and it is the cause of both personal and social problems in endemic regions.<sup>3</sup>

After being bitten by an infected sand fly, the nodules, papules or ulcers begin to develop.<sup>2</sup> The parasites begin to multiply within the macrophages and

then generally heal, leaving a scar. Histopathologically, hyperkeratosis, parakeratosis, follicular plugging and degeneration of the basal layer are seen in the epidermis, parasites are not found, or are rarely found in epidermal keratinocytes. In the dermis, there is the infiltration of lymphocytes, histiocytes and plasma cells. While in early lesions the number of parasites is concentrated, it is difficult to find any parasites at all in older lesions.<sup>8,9</sup>

In a study by Rawlins et al.<sup>10</sup> including 185 cases in South America and Guyana, they showed that 43.8% of their biopsy materials had parasites. Kubba et al.<sup>11</sup> found that *L. major* was the influencing factor of the 475 cases in Saudi Arabia; 50-80% was obtained by smear, 70% by biopsy and 50% by culture, regardless of the fact that all three methods were used; they did not find parasites in 10-20% of cases. In our study, we evaluated forty cases that the leishmania parasites had been identified in biopsy and/or smear. It is useful if biopsies and touch imprints are performed at the same time. Alternatively, drawing them with a lancet, spreading the resultant material and dyeing it with Giemsa stain have allowed us to identify the parasites more easily and therefore, reach to a correct conclusion. In addition, granulomas without necrosis, abortive granulomas as in the form of histiocyte groupings, infiltration of dermis with plasma cells and eosinophil should alert us to look for the parasites as leishmaniasis. In our study, the cases where biopsies and smears were performed at the same time were excluded. In addition, we could not obtain enough clinic information about the earlier smears.

The differential diagnosis of CL includes tropical and traumatic ulcers, reactions to foreign bodies, infected insect bites, impetigo, myiasis, mycobacterial and fungal infections, sarcoidosis and tumors.<sup>12,13</sup>

For accurate diagnosis of CL, apart from clinical history and typical appearance of the lesion, demonstration of the parasite in tissues and on smears, its production in cultures or detection by PCR are also recommended. Skin tests, serological, immunoperoxidase and immunofluorescent methods can also help in putting a diagnosis.<sup>1-3,14,15</sup> In most cases, using these methods may not be very beneficial, because monoclonal antibodies are species specific and antibody concentrations are so low in serologic

tests.<sup>12,13</sup> In a study by Schubach et al.<sup>16</sup> including 88 cases, leishmania parasites were shown in 30.2% of cases by the histopathological examination, 28.2% by using imprints, 43.4% by using cultures, 41.4% by using immunofluorescence and 58.5% by using immunoperoxidase.

If the diagnosis of CL is established too late, growth in lesions and scarring, bacterial super infections and mucosal leishmaniasis develop.<sup>12</sup> In treatment, intralesional or systemic treatment with meglumine antimoniate or sodium stibogluconate and cryotherapy are used.<sup>1-3,12-14</sup>

Today, protection from increasing instances of this endemic illness remains at the forefront. Fighting to the vectors, educating the public and to recognize clinical and morphological features of the infection by clinicians and pathologists remain of utmost importance.

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