

Original Article

Histopathological spectrum of cutaneous leishmaniasis in North West Frontier Province

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Abstract *Objectives* The objective of the study was to determine various histological spectrum of cutaneous leishmaniasis in NWFP and to detect if there were any other useful histological findings in addition to the detection of amastigotes, which could help in the diagnosis.

Materials and methods Total number of patients included in the study was 36. These patients were referred from various areas of NWFP. All except one were males. Their age ranged between 8-60 years. The duration of illness was from 3-32 weeks. A skin biopsy was performed from the most typical lesion from each patient. The biopsy slide was stained with H&E stain and viewed under a microscope by a single expert. The histopathological findings were noted in accordance to uniform criteria.

Results The confirmation of the diagnosis was done by the detection of amastigotes (LT bodies) in the slides. They were detected in 11 out of 36 patients (30.55%). The other most significant finding detected in all the slides was the presence of a mixed inflammatory cell infiltrate in the superficial dermis.

Conclusion It is concluded that the chances of picking up of amastigotes in the biopsy slides is not high. Hence, the presence of dense mixed inflammatory cell infiltrate in superficial dermis must be considered as a diagnostic feature in the absence of LT bodies.

Key words

Cutaneous leishmaniasis, histopathology, LT bodies

Introduction

Definitive diagnosis of Leishmania infection is necessary before the start of chemotherapy. The laboratory diagnosis of cutaneous leishmaniasis requires demonstration of parasites in smear, biopsies, or by isolation of the organism in culture medium or in experimental animal. Many other methods for demonstration of

parasite (histochemical and immunohistochemical) or for detecting antibodies against leishmania (serologic) have been described. Many advances have been made in these areas, but the methodology and the technology involved in immunohistochemistry and serology remain outside the reach of the standard laboratories. Both in developed and less developed countries, the laboratories still rely on demonstration of parasites in the smear stained with Giemsa and on biopsy specimens processed and stained with haematoxylin and eosin (H&E).¹

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Skin biopsy for histopathology is the most frequently performed investigation in Pakistan. The positive report requires the presence of amastigotes in the slides. The chances of picking up amastigotes in histopathology slides are not high, so it is important to identify other histopathological findings as well which can contribute to the diagnosis.

Materials and methods

Study design

This was a non-interventional, descriptive study.

Objectives of the study

There were two objectives of this study:

1. Firstly, to detect various histopathological features in the patients of cutaneous leishmaniasis in North West Frontier Province, and to see if the results are similar or different from the results from other studies.
2. Secondly, to determine if there are any other histopathological findings in addition to the detection of LT bodies in the definitive diagnosis.

Study population

The study population comprised army personnel and their relatives who were referred to the skin department of Combined Military Hospital, Peshawar, from Kohat, Bannu, DI Khan, and North and South Waziristan agencies. Total number of patients included in the study was thirty-six (n=36). All the patients except one were males. Their ages ranged between 8-60 years (Mean 31.97 years). The duration of

the disease was from 3-32 weeks (Mean 10 weeks). The centre of trial was skin and histopathology department of Combined Military Hospital, Peshawar.

Inclusion criteria

The study included patients of all ages with one or more nodular, ulcerated or crusted plaques over exposed areas of the body since at least 3 weeks (presence of satellite lesions or sporotrichoid spread further supported the diagnosis). A history of contact of the disease in a known endemic area was also considered as one of the inclusion criterion.

Exclusion criteria

The study excluded very young and very old patients, patients who did not agree to be included in the trial and the patients with a doubtful clinical lesion. Those patients who had no history of travel to an endemic area and those who received some definitive treatment for their disease were also excluded.

Record keeping

A typed pro forma was prepared, which was used for every patient. It included name, age and sex of the patient, the duration of illness, area of contact of the disease and the histopathological findings in accordance with uniform criteria.

Histopathological examination

Skin biopsy was performed from the edge of the most representative lesion in every patient. The specimen was sent to histopathology department in formalin. The formalin-fixed specimens were paraffin embedded after going through a series of processing i.e. fixation, dehydration,

clearing and impregnation. The section cutting was done by rotatory microtome (Model AS-325 Shandon), followed by haematoxylin and eosin staining. The slides when prepared were examined by a single microscope (Model CH-20 Olympus), and by only one histopathologist in consultation with a dermatologist.

Results

Following were the results of this study:

1. Leishmania trophozoite (LT) bodies were picked up in the histopathology slides in 11 out of 36 patients (30.5% yield).
2. A feature seen in all histopathology slides was the presence of dense, mixed inflammatory cell infiltrate, composed of lymphocytes, histiocytes and plasma cells in the superficial dermis (100% yield).
3. Neutrophils were a part of this infiltrate in the histopathology slides of 26 patients (72.2%). Among these 26 slides, neutrophilic abscesses were seen in 14 patients (38.9%).
4. Epidermal ulceration was seen in histopathology slides of 28 patients (77.8%), almost always associated with the presence of neutrophils in the infiltrate.
5. Epidermal hypertrophy was seen in all the slides (100%).
6. Epithelioid cells were in histopathology slides of 20 patients (55.5%), and epithelioid cell granulomas in 16 patients (44.4%). Langhans giant cells were noted as a part of granulomas in 11 patients (30%) and caseation necrosis

within the granulomas in 7 (19.4%) patients.

Clinico-pathological correlation

In wet and ulcerated lesions, the infiltrate was mainly mixed, and there were more chances of picking up of amastigotes in the histopathological slides. In dry and nodular lesions, the infiltrate was again mixed but there was a greater tendency of granulomas formation with less chances of picking up amastigotes.

Histological patterns

Four definitive histological patterns were identified in this study. These were:

1. Mixed inflammatory cell infiltrate with LT bodies and no granulomas (7 [19.5%] patients) [**Figure 1**].
2. Mixed inflammatory cell infiltrate with LT bodies and presence of epithelioid cell granulomas (4 [11.1%] patients).
3. Mixed inflammatory cell infiltrate, with no LT bodies and the presence of epithelioid cell granulomas (12 [36.1%] patients) [**Figure 2**].
4. Mixed inflammatory cell infiltrate with no LT bodies and no granulomas (13 [33.3%] patients) [**Figure 3**].

Discussion

From a specific area of the country, 4 definitive patterns emerged. In a previous study published in Pakistan,² the histopathological findings were also placed in four groups. In two of the groups (76% of the patients), there was mixed inflammatory cell infiltrate, in 12% there was chronic inflammatory infiltrate without granuloma

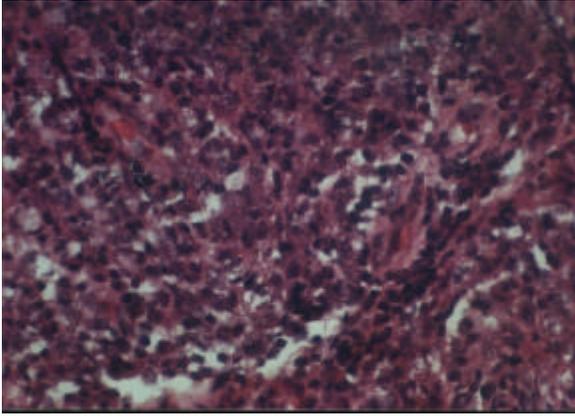


Figure 1: Mixed inflammatory cell infiltrate with LT bodies and no granuloma.

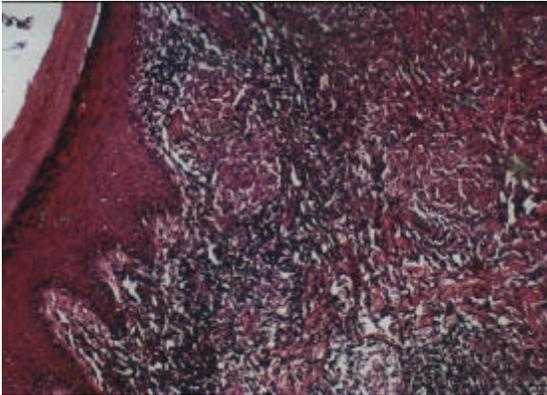


Figure 2 Mixed inflammatory cell infiltrate with epithelioid cell granulomas and no LT bodies.

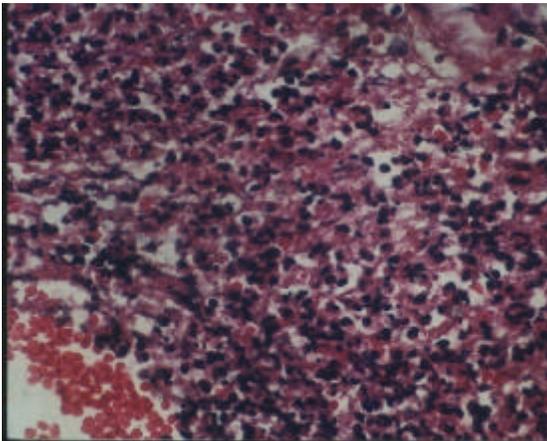


Figure 3 Mixed inflammatory cell infiltrate consisting of Lymphocytes, neutrophils, plasma cells and histiocytes. There are no LT bodies or granulomas.

and in 12 % there were only tuberculoid granulomas. In our study, there was no pure

tuberculoid pattern or pure mononuclear infiltrate.

Despite the fact that histopathological examination of skin biopsy is the most frequently performed investigation in the patients of leishmaniasis, the chances of picking up LT bodies are not high. In our study LT bodies were detected in 11 out of 36 patients (30.5%). The results in other studies are much the same as ours. Bhutto *et al.*³ detected LT bodies in 365 out of 1210 patients registered (diagnostic yield was 30.2%). Sharquie *et al.*⁴ found amastigotes in 18 out of 60 patients (30% yield). In the study by Weigle *et al.*⁵ 23 slides were positive for LT bodies among the total 165 patients (13.9% yield). Azedah *et al.*⁶ detected amastigotes in 64 out of 117 slides (54.7% yield) and Simeen *et al.*⁷ reported 76% yield by picking up amastigotes in 38 out of 50 examined slides. This comparison is graphically shown in **Figure 4**.

After seeing the above comparisons the first question which arises is that if the percentage yield of amastigotes in skin biopsy is so low, is such an invasive procedure, which is also costly in our setting, justifiable in the diagnosis? Secondly, in the absence of demonstrable LT bodies can there be any other histological feature, which can contribute to the confirmation of the diagnosis of cutaneous leishmaniasis?

Features like epidermal hyperplasia, epidermal ulceration, granuloma formation, Langhans cells and mononuclear or polymorphonuclear infiltrate occurs in other related diseases e.g. lupus vulgaris, dermatophyte infection, etc. A feature that

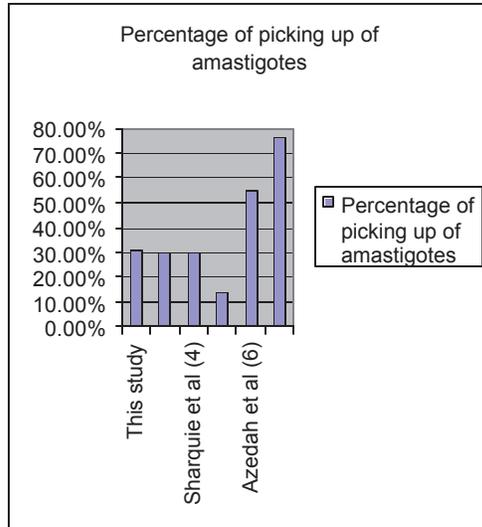


Figure 4 Comparison of percentage of picking up of amastigotes in various studies

was present in all the slides in our study, no matter what the duration of the illness, was the presence of a dense and superficial mixed inflammatory cell infiltrate comprising of mainly lymphocytes, plasma cells and histiocytes. Neutrophils and epithelioid cells were also a part of this infiltrate in most of the cases. Hence, this mixed inflammatory infiltrate must be taken as a definitive diagnostic feature in the absence of demonstrable LT bodies.

Conclusion

Chances of picking up amastigotes in histopathology slides are not very high. A constant finding in our study was the presence of a dense mixed inflammatory cells infiltrate in the superficial dermis. This feature is not present in any other chronic

granulomatous disease; hence, this should be taken as a diagnostic feature in the absence of LT bodies.

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