Research Article

An institution-based observational study to identify sensitive histopathological parameters in leprosy

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Abstract

Background: Spectral concept of leprosy has evolved from the histopathology of leprosy skin lesions.

Objective: To identify sensitive histopathological markers of leprosy, to enhance the specificity of clinical diagnosis of leprosy, and to continue research and training in leprosy in postelimination era.

Material and Methods: This study was carried out in a state government referral center for leprosy from April 2009 to March 2010. Paraffin sections of biopsies were stained with hematoxylin and eosin, Ziehl–Neelsen, and Fite stains, examined, and classified histopathologically according to Ridley–Jopling scale. All new cases of leprosy diagnosed based on presence of at least two of the three cardinal features of leprosy were included. Released from treatment cases, partially treated cases, and those with lepra reactions were not included in this study.

Result: Fifty-nine skin biopsies of newly diagnosed untreated leprosy cases over a period of 1 year were included. Of the total 59 patients, the distribution of various types of leprosy is as follows: polar tuberculoid (TTp) leprosy, 18.33% (n = 11); borderline tuberculoid (BT) leprosy, 28.33% (n = 17); mid-borderline leprosy, 6.66% (n = 4); borderline lepromatous (BL) leprosy, 30% (n = 18); polar lepromatous leprosy, 5%(n = 3); histoid leprosy, 5% (n = 3); indeterminate leprosy, 5% (n = 3). Of the 11 patients with TTp leprosy, 81.8% (n = 9) showed mature epithelioid cell granuloma; of the 17 patients with BT leprosy, 94.11% (n = 16) showed immature epithelioid cell granuloma. Of the 18 patients with BL leprosy, 83.33% (n = 16) showed macrophage granuloma. Of the 28 patients on tuberculoid pole, 82.16% (n = 31) showed lymphocyte predominant infiltrate. Of the 32 patients on lepromatous pole, 84.37% showed macrophage predominant infiltrate. Wide Fite staining was positive in 14.28% (n = 4) on tuberculoid pole and in 87.5% (n = 28) on lepromatous pole.

Conclusion: Histopathology remains the only practical and viable tool for diagnosis of specific subtype of leprosy. Mature epithelioid granuloma is the most sensitive indicator for tuberculoid leprosy, and grenz zone is the most sensitive indicator of BL leprosy. Diffuse infiltration of the dermis except for narrow subepidermal zone is the most sensitive indicator of lepromatous leprosy.

KEY WORDS: Granuloma, grenz zone, spectral concept

Introduction

Leprosy present in many ways, and emphasis for diagnosis of leprosy largely remains on clinical presentation. Attempts to develop cheap, highly sensitive, and specific serological test have yielded poor results with very limited application of such tests in community-based approach to leprosy. Histopathology remains the technique to establish and confirm the diagnosis of leprosy.

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Methods and Materials

The study was carried out on the skin biopsies from untreated cases of leprosy seen in the Department of Dermatology and reported in the histopathology section of the Department of Pathology at a tertiary center from April 2009

to March 2010. All new cases of leprosy diagnosed based on presence of at least two of the three cardinal features of leprosy were included.

Hematoxylin and eosin-stained sections of 4-mm punch biopsy was performed from the appropriate sites and following parameters were noted. Fite-Foraco staining was performed on the histopathological sections and examined for: (1) presence of granuloma—its location whether epidermal, dermal, or periappendiceal, mature or immature type, and predominant cell type of granuloma whether epithelioid or macrophage; (2) cellular infiltrate: predominant cell type, presence or absence of Langhans giant cells or foamy macrophages, and the distribution; and (3) other histopathological features such as obliteration of dermal nerves by granuloma, erosion of epidermis by granuloma, and presence or absence of grenz zone.

The sections stained with Ziehl–Neelsen (ZN) stain and modified Fite stain were examined for lepra bacilli in all cases. Histopathological findings were graded into polar tuberculoid (TT) leprosy, borderline tuberculoid (BT) leprosy, mid-borderline (BB) leprosy, borderline lepromatous (BL) leprosy, and lepromatous leprosy (LL) according to the scale by Ridley and Jopling. Sections showing scattered nonspecific lyphohistiocytic infiltration with cellular reaction within dermal nerve or presence of bacilli in subepidermal zone/arrector pilorum muscle/dermal nerve were classified as indeterminate leprosy.

Biopsy that did not include the full depth of dermis together with a portion of subcutaneous fat were considered as inadequate, not classified histopathologically, and were excluded. Released from treatment cases/partially treated cases/those with lepra reactions were not included in this study.

Results

This study was done on skin biopsies of 59 clinically diagnosed untreated case of leprosy of which 34 were male and 25 female subjects. Their age ranged from 11 to 67 years, with majority of them in the age group of 20–40 years. On the basis of the clinical findings, they were subdivided into subtypes of leprosy: BL leprosy, 29.03% (n = 18); BT leprosy, 27.14% (n = 17); TT leprosy, 17.24% (n = 11); and LL, 9.46% (n = 6), of which 3 cases were of histoid leprosy, Mid-borderline leprosy, 6.45% (n = 4), and indeterminate leprosy, 5.08% (n = 3), were also found [Figure 1].

In this study, BL leprosy was the most common type of leprosy. In the TT leprosy, perivascular and periadnexal infiltrates seen in 100% of cases were the most common histopathological feature, followed by lymphocyte predominance of infiltrate (90.90%), mature epithelioid granuloma (81.8%), immature epithelioid granuloma (18.18%), and erosion of epidermis by granuloma (9.09%). Acid-fast bacilli (AFB) were seen in 9.09% cases by modified ZN stain method and Fite stain [Figure 2].

In BT leprosy, immature epithelioid granuloma (94.11%), perivascular and appendiceal infiltrate (94.11%), lymphocyte predominance of infiltrate (76.47%), obliteration of nerve by granuloma (41.17%), and mature epithelioid granuloma (23.52%) were observed. AFB were seen in 11.76% cases by modified ZN stain method and in 17.64% cases by modified Fite stain [Figure 3].

In mid-borderline leprosy, grenz zone in 75%, AFB in 50% cases by ZN stain and 75% cases by modified Fite stain, macrophage predominance of infiltrate in 75% cases, perivascular and periappendiceal infiltrate in 50%, immature

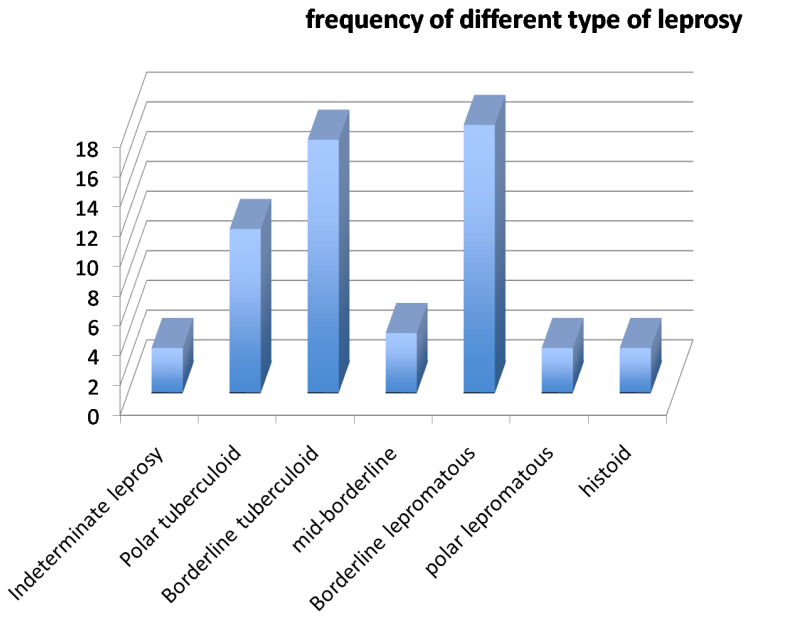

Figure 1: Frequency of various subtypes of leprosy observed in this study.

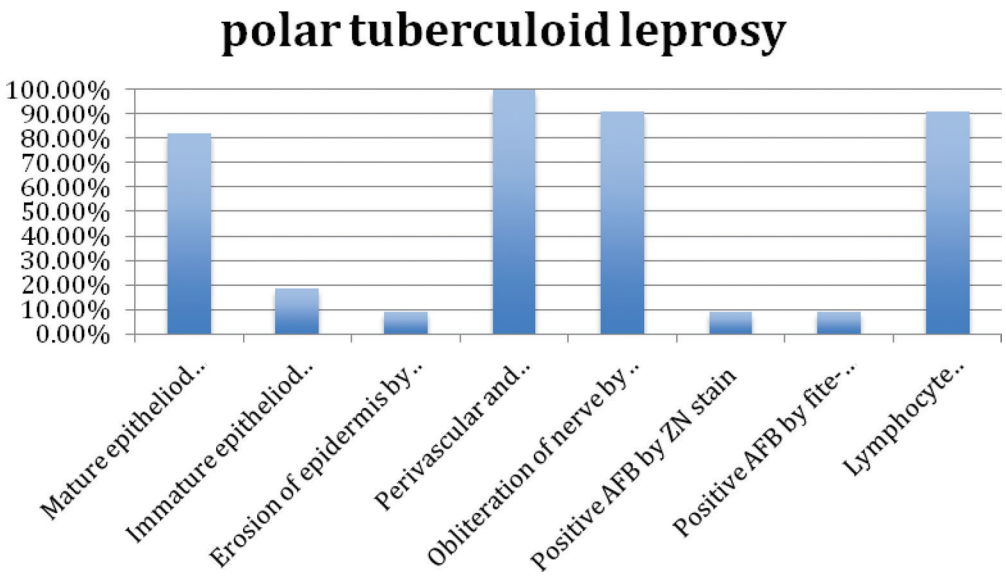

Figure 2: Frequency of histopathological parameters observed in polar tuberculoid leprosy.

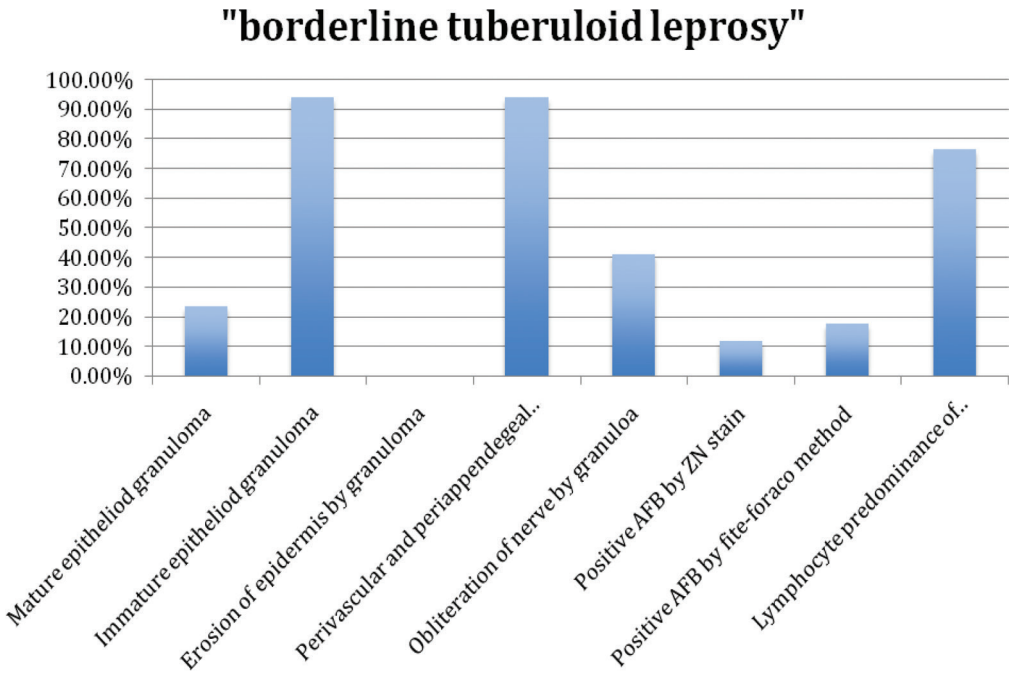

Figure 3: Frequency of histopathological parameters observed in borderline tuberculoid leprosy.

epithelioid granuloma and obliteration of nerve by granuloma in 33.33% of cases, and lymphocyte predominance of infiltrate, macrophage granuloma, and presence of Langhans cells each in 25% of cases were seen.

In BL leprosy, diffuse infiltration of the dermis, except for narrow subepidermal zone (grenz zone), was seen in 88.88% cases, in addition to macrophage predominance of infiltrate in 83.33% cases and AFB that stained positively in 72.22% cases by modified ZN stain, while the sensitivity of bacilli detection increased to 83.33% when stained with modified Fite stain. Langhans giant cell were seen in 77.77% of cases. Obliteration of nerve by granuloma and ill-defined macrophage granuloma was seen in 16.66% cases [Figures 4 and 5].

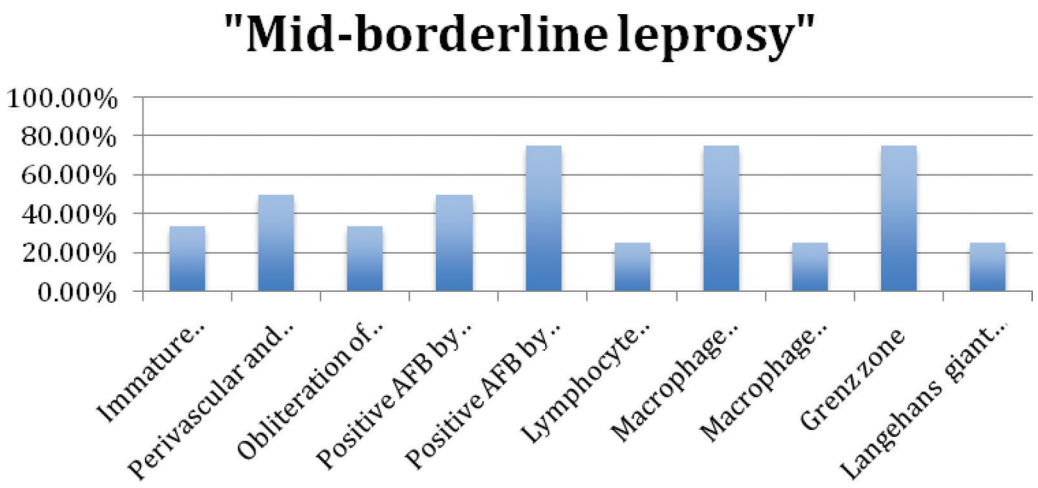

Figure 4: Frequency of histopathological parameters observed in mid-borderline leprosy.

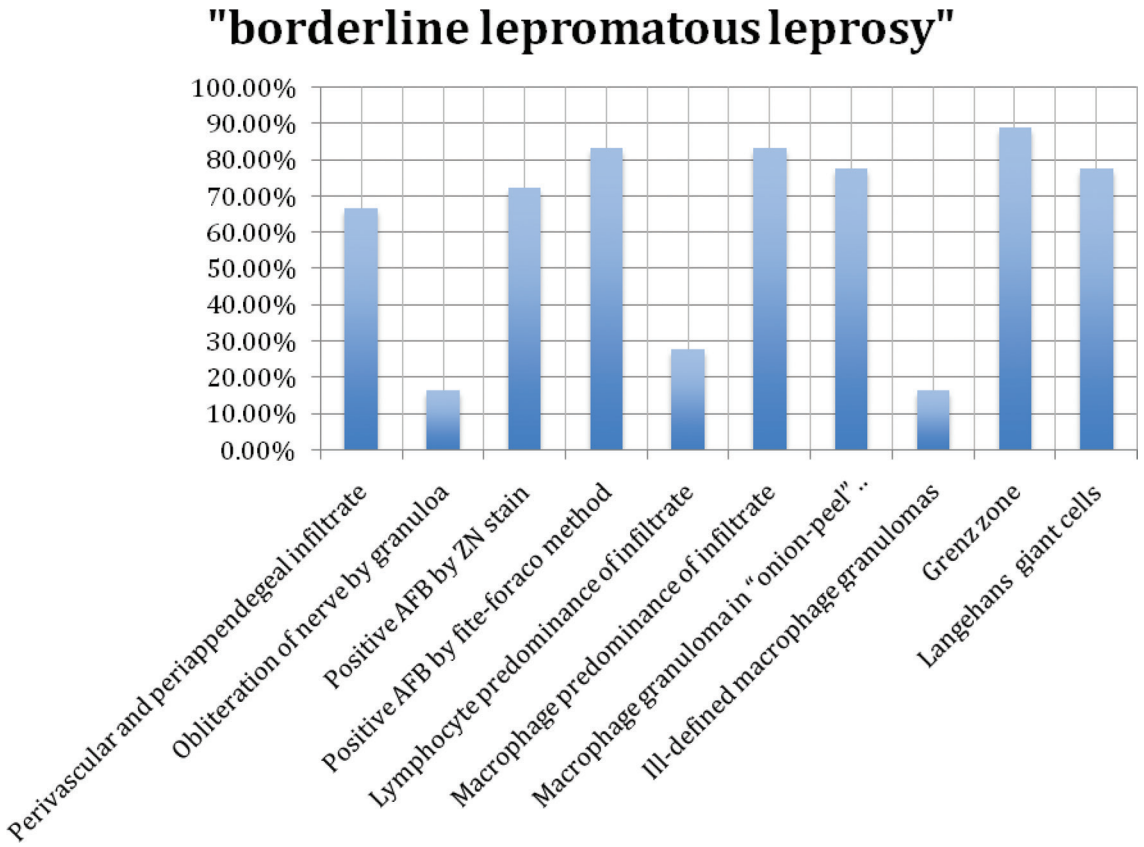

Figure 5: Frequency of histopathological parameters observed in borderline lepromatous leprosy.

In LL, grenz zone with pan dermal infiltrate was seen in 100% cases, while histoid leprosy showed spindle-shaped tumor-like infiltrate of histiocytes forming whorls and/or interlacing bands in the dermis. Presence of foamy macrophages in 66.66%, macrophage granuloma in 66.66%, and Langhans cells in 66.66% cases was observed. All cases stained positively for AFB with modified ZN stain and modified wide Fite stain [Table 1].

In indeterminate leprosy, all the three (100%) cases showed mild nonspecific perivascular and periadnexal

Table 1: Comparison of histopathological parameters observed in lepromatous leprosy and histoid leprosy

Type of leprosy	Lepromatous leprosy, *N* = 3	Histoid leprosy, *N* = 3
Pan dermal infiltrate	Diffuse infiltration of the dermis except for narrow subepidermal zone	Spindle-shaped tumor-like infiltrate of histiocytes forms whorls and/or interlacing bands in the dermis
Obliteration of nerve by granuloma	1 (33.33%)	—
Positive AFB by ZN stain	3 (100%) fragmented bacilli	3 (100%) uniformly staining rod-shaped bacilli
Positive AFB by Fite-Faraco method	3 (100%)	3 (100%)
Macrophage predominance of infiltrate	3 (100%)	3 (100%)
Presence of foamy macrophages	2 (66.66%)	0
Macrophage granuloma in "onion-peel" configuration	2 (66.66%)	0
Grenz zone	3 (100%)	0
Langhans giant cells	2 (66.66%)	0

infiltrate and lymphocyte predominant infiltrate with presence of histiocytes, while one of the three (33.33%) cases showed obliteration of dermal nerve by the infiltrate.

Discussion

A disease such as leprosy needs an appropriate classification because of its varied manifestations. The classification by Ridley and Jopling[1] is the most widely recognized classification by research workers, which is fundamentally grounded on immunity but has been connected with clinical, histopathological, and bacteriological findings. In spite of the availability of such a precise classification, many variations between the clinical and histopathological features have been observed in the leprosy cases.

Diagnosis of majority of leprosy cases is possible without histopathological examination. Histopathological evaluation of a biopsy specimen provides a reliable aid in reaching confirmatory diagnosis and its subtypes, differential diagnosis and prognosis of the disease, and assessment or regression of the disease in the patient under treatment and for research.[2,3]

The inflammatory cells that are peripheral in nature and compact in collection with predominance of mononuclear cells can be described as granulomas. The persistence of a non-degradable product or hypersensitivity responses can result in the formation of granulomas.[4]

The various clinical forms by which leprosy establishes are complemented by particular histopathological picture. Thus, histopathology reveals epithelioid cells, Langhans giant cells, and lymphocytes in toward the TT end of the spectrum and while foamy macrophages are abundant toward LL end of spectrum.[1]

The objective of the study was to identify the most sensitive histopathological parameter for the presented clinical type of leprosy.

Similar to our study, various studies reported better demonstration of AFB in biopsy than in slit-skin smear (SSS).[5,6] Bhusan et al. found significant number of positive cases in biopsy, which constituted 65 (46.09%) cases, while SSS positive only in 43 (30.05%) cases. Both SSS and Fite stain were negative in indeterminate and tuberculoid leprosies as in our study, and they concluded that demonstration of bacilli in biopsy as most sensitive and effective method, especially in pauci-lesional patient. AFB are better demonstrated in biopsies than in SSS because of the presence of AFB in deep reticular dermis where they remain inaccessible to SSS.[7]

On the basis of this study, in the TT leprosy, perivascular and periadnexal infiltrate is the most sensitive histopathological feature, followed by lymphocyte predominance of infiltrate, mature epithelioid granuloma, immature epithelioid granuloma, and erosion of epidermis by granuloma.

It is a known fact that various clinical forms by which leprosy establishes are accompanied by particular histopathological picture. Thus, toward TT end of the spectrum, histopathology shows epithelioid cells, Langhans giant cells, and lymphocytes, and toward LL end of spectrum, more foamy macrophages are observed.[9]

Leprosy is diagnosed based on various clinical parameters, which include thorough examination of skin lesion and examination of peripheral nerve and skin smear. Diagnosis only on clinical basis is not accurate in some early and borderline cases of leprosy; hence, such cases need to be confirmed by histopathological examination. The correlation between clinical and histopathological features plays an important role for early diagnosis. If discrepancy is seen, advanced findings such as toward lepromatous pole must be considered with utmost importance, and it is classified and treated consequently so that insufficient treatment can be avoided.

Histopathological Differential Diagnosis

Differentiation of tuberculoid leprosy from other granulomatous dermatitides is essential. Exclusion of cutaneous tuberculosis is also a very important differential diagnosis. Tuberculoid leprosy reveals flat epidermis, while tuberculosis shows hyperplastic epidermis. The granuloma arrangement in leprosy reveals an oblong pattern of neurovascular bundles similar to the granuloma unlike tuberculosis, which

shows an intense and sometimes lichenoid pattern of the chronic granulomatous infiltrate. The infiltrate in tuberculosis spares the dermal nerve twigs when observed. The decisive finding of leprosy is the presence of granuloma or AFB in the nerve. Owing to the presence of fibrinoid necrosis in both the scenarios, tuberculoid leprosy may be identified as cutaneous sarcoidosis. Paucity of lymphocytes, more confluence, and fibrosis around the granuloma are the characteristic features of the granulomas of sarcoidosis. Exclusion of other granulomatous lesions such as leishmaniasis or granulomatous post-kala-azar dermal leishmaniasis can be done by the identification of Leishman–Donovan bodies and frequent presence of plasma cells. BL leprosy and pure LL may be muddled owing to the presence of with histiocyte-rich lesions such as xanthomas; however, this diagnostic dilemma can be solved by the demonstration of AFB in these lesions.

Conclusions

Lesions classified by clinical parameters consider only their gross appearances; however, the classification based on histopathological parameters is well defined and accurate and considers the immunological manifestations that facilitate the successful diagnosis of leprosy by bridging the pitfalls. Extensive search over PubMed showed only 155 citations related to histopathological changes in leprosy. The average age of the researchers was 40–45 years. This shows lack of research activity in young individuals and further need to be encouraged to research in this regard.

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