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**Original Research Article**

# To study Characterizing of Early Leprosy by routine histopathology Immunochemistry and by methods of Molecular Biological

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**Abstract**

**Background & Methods:** The aim of the study is to study Characterizing of Early Leprosy by routine histopathology Immunochemistry and by methods of Molecular Biological.

**Results:** The lesions were found to be mostly in the extremities (44/50, 88%) with the upper extremities being more frequently involved. Nerve thickening was observed to be present in 26% of the cases, with cutaneous nerve involvement being seen in 5 (10%) and peripheral nerve trunk involvement in 8 (16%). The skin smear was negative for AFB in all, and the Mitsuda lepromin reaction was positive in 18/30 (60%) of the cases studied.

**Conclusion:** The present study, 100 cases of clinically diagnosed early leprosy (Idt leprosy, early TT/BT) and, 90 cases of clinically suspect leprosy were studied from the viewpoint of histological diagnosis. Homogeneity within the two groups was achieved by selecting only untreated cases and by defining the features required to make the clinical diagnosis. Biopsies were taken from the lesion peripheries, fixed in 10% buffered formalin, and, processed for routine histopathological examination. The histological diagnosis of leprosy, using specific defined criteria was made in (36%) in the clinical category of early leprosy, and in (24.44%) in the clinical category of suspect leprosy. These findings are consistent with the low (and variable) degree of histological confirmation reported in several studies in the chosen categories of cases.

**Keywords:** Leprosy, histopathology, Immunochemistry and Molecular Biological.
**Study Design:** Observational Study.

## 1. Introduction

Leprosy is an ancient disease caused by Mycobacterium leprae or Mycobacterium lepromatosis infection, which mainly impairs skin and peripheral nerves and can even result in disability[1]. The pathogens of leprosy have accompanied and affected humans for more than 4,000 years, and over 200,000 new cases of leprosy are still reported each year worldwide despite the application of multidrug therapy by the Word Health Organization. Because of the severe consequences caused by leprosy, including appearance changes and disabilities, leprosy is

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still a significant public health issue, especially in countries such as Brazil, India, and Indonesia, where the disease is still prevalent[2].

As the clinical manifestation of leprosy presents as a spectrum, it has long been considered an attractive model by immunologists to study the interaction between immune response and infection. Based on the different immune responses observed in patient lesions, leprosy can be categorized into five groups: tuberculoid (TT), borderline tuberculoid (BT), borderline borderline (BB), borderline lepromatous (BL), and lepromatous (LL) [3]. The World Health Organization classifies leprosy clinically as multibacillary and paucibacillary, according to the number of skin lesions and nerve involvement [4]. During the chronic infectious course, the immune-mediated acute inflammatory episodes called leprosy reactions frequently occurred. Leprosy reactions can be classified into two major types: type 1 reaction (T1R) or reversal reaction occurring mostly in unstable borderline patients (BT, BB, BL) and LL patients and type 2 reaction (T2R) or erythema nodosum leprosum (ENL) occurring mostly in BL and LL patients [5]. Therefore, leprosy is considered as an ideal disease model by immunologists to investigate the interrelation between pathogen load in infection and the differential immune responses of the host. Nevertheless, the pathogenesis of leprosy remains ambiguous due to the lack of an ideal animal model for this disease.

## 2. Material and Methods

Present Study was conducted at Maharishi Markandeshwar College of Medical Sciences and Research, Ambala for 01 Year on 100 patients 82 adult & 18 paediatric.

**CLINICAL CASE SELECTION**

Cases were chosen from the patients attending the out-patient departments. Only untreated cases, presenting themselves for the first time were included. The clinical categories of the cases included were

**(a) Early Tuberculoid or Borderline Tuberculoid leprosy.**

Wherein the lesions were erythrochromic or hypochromic, with defined margins, flat or partly elevated, showing central clearing, with or without

**(b) Indeterminate leprosy:**

Wherein the lesions were flat, hypopigmented, or, erythrochromic, with vague ill-defined margins and showing sensory deficit. Though sensory changes may not always be present in Indeterminate leprosy, for the initial clinical level of case selection where no corroborative findings (such as presence of AFB or nerve infiltration) are available, sensory deficit was made a pre-requisite for clinically diagnosing Indeterminate leprosy. If peripheral nerve trunk thickening was present the diagnosis was considered to be BT leprosy.

Immunostaining was performed on paraffin sections using an indirect immunoperoxidase procedure incorporating either the Avidin Biotin, or, the Streptavidin Biotin system. The primary -antigen detecting- antibody used was anti- M bovis BCG (DAKO BO124); the binding of this antibody to mycobacterial antigens within the tissue was detected by the sequential application of a biotinylated secondary antibody followed by horseradish peroxidase conjugated Avidin-Biotin or Streptavidin-Biotin. End product visualization was achieved using 3'3' diaminbenzidine as the chromogen.

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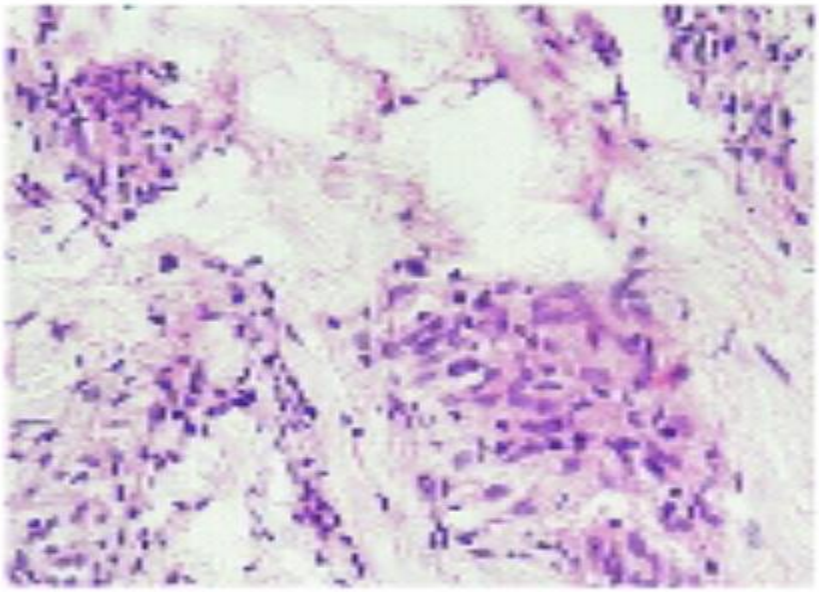

Figure 2. A: FNAC of inguinal lymph node showing ill-defined epithelioid cell granulomas and foamy histiocytes, in background of lymphoid cells in various tages of maturation (Leishman Giemsa, x400)

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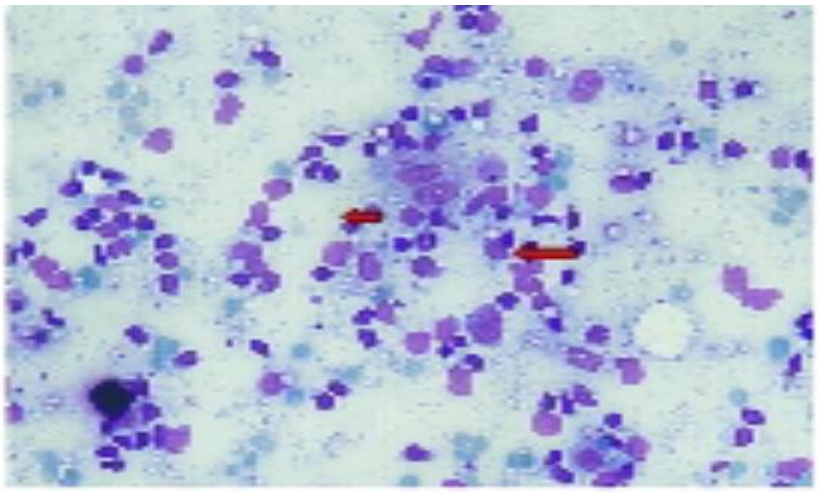

Figure 2. B Another area higher magnification showing numerous foamy histiocytes with few epithelioid cells in a lymphoid background.

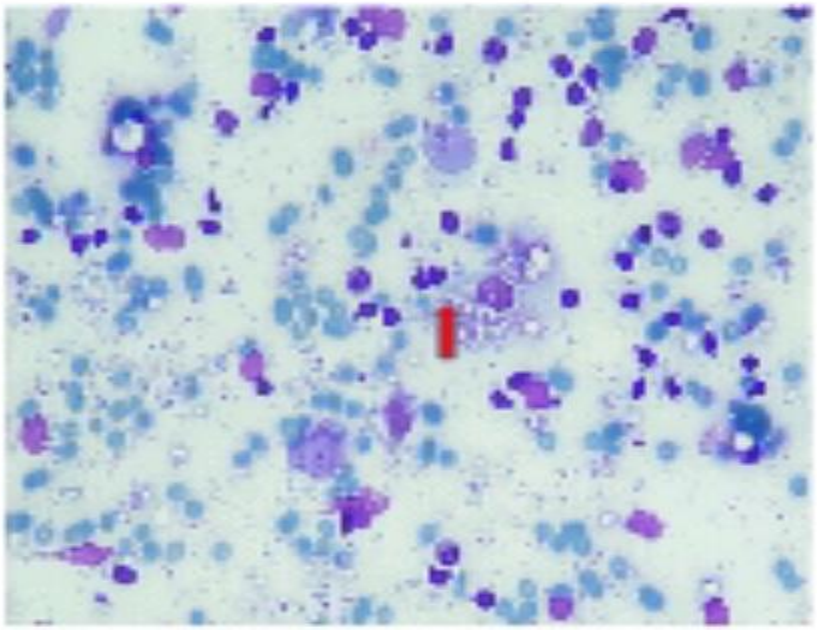

Figure 2.C Foamy histiocytes in the Centre showing negative images of leprae bacilli (x400)

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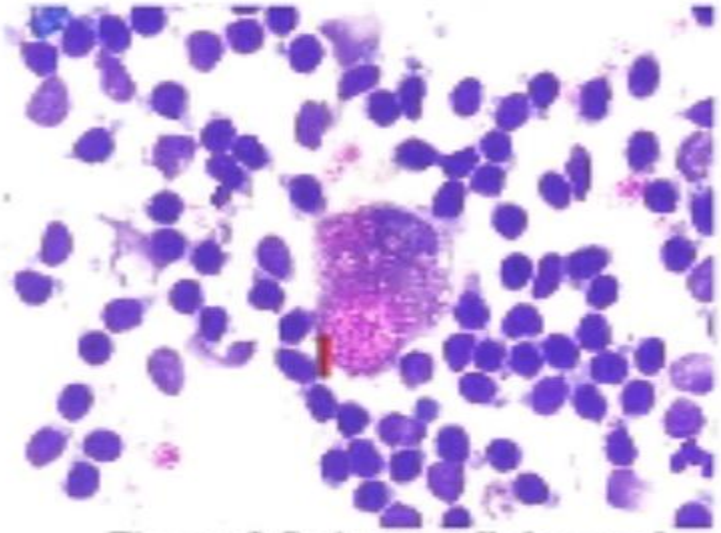

Figure 2.D: Intracellular and extracellular lepra bacilli as globi in foamy macrophages (ZN x1000)

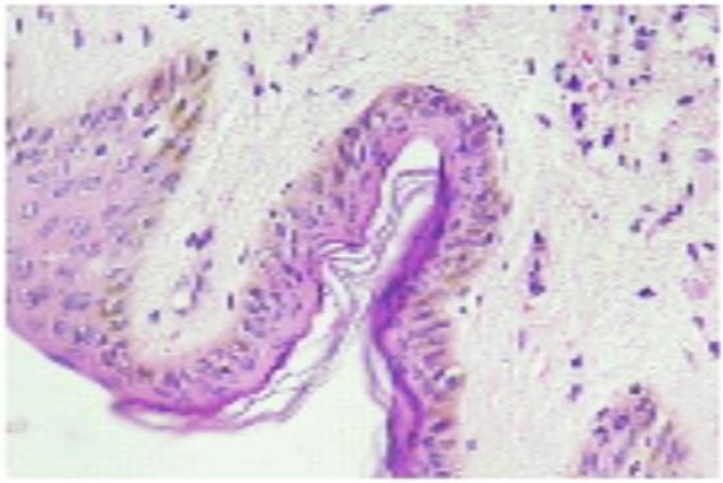

Figure 3.A: Punch biopsy from skin ɛsions showing Grenz zone (H and E; x400)

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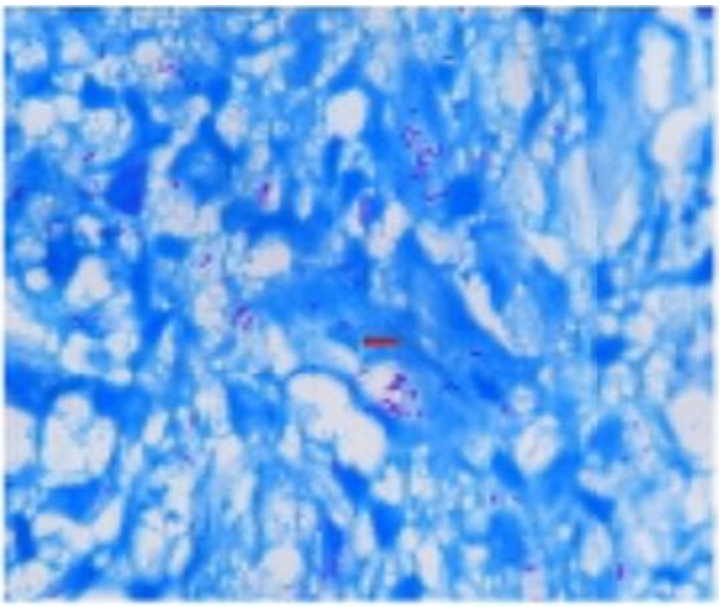

Figure 3.B: Foam cells showing lepra bacilli (ZN, x1000)

## 3. Result

**Table 1: Gender Distribution**

| S. No. | No. | Percentage |
|---|---|---|
| **Male** | 88 | 88 |
| **Female** | 12 | 12 |
| | | |

Mean Age – 28.6

**Table 2: Symptoms Duration**

| S. No. | No. | Percentage |
|---|---|---|
| **>12** | 57 | 57 |
| **<12** | 43 | 43 |
| | | |

It was observed that the cases were predominantly male adults with their ages ranging from 14 to 60 years. The disease duration too, showed a wide range (1 60 months) in keeping with the variable progression and self- regression exhibited by this category of leprosy. However, the duration in (43%) of the cases was less than 12 months.

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**Table 3: Lesion Distribution**

| S. No. | No. | Percentage |
|---|---|---|
| **Extremities** | 88 | 88 |
| **UE:LE** | 33:11 | |
| | | |

The lesions were found to be mostly in the extremities (88%) with the upper extremities being more frequently involved. Nerve thickening was observed to be present in 26% of the cases, with cutaneous nerve involvement being seen in (10%) and peripheral nerve trunk involvement in 16 (16%). The skin smear was negative for AFB in all, and the Mitsuda lepromin reaction was positive in (60%) of the cases studied.

**Table 4: Histopathology Data**

| S. No. | No. | HP Diag. Leprosy |
|---|---|---|
| **Early Leprosy** | 100 | 36% |
| **IDT Leprosy** | 26 | 30% |
| **Early TT/BT** | 74 | 37.8 |
| **Suspect Leprosy** | 90 | 22.2% |

**Table 5: Immunostaining Data**

| S. No. | No. | No. of Positive | Percentage Positivity (%) |
|---|---|---|---|
| **Early Leprosy** | 64 | 22 | 34.37 |
| **IDT Leprosy** | 18 | 06 | 33.33 |
| **Early TT/BT** | 46 | 08 | 34.78 |
| **Suspect Leprosy** | 70 | 12 | **17.14** |

Their locations too were similar with the nerve being the most common site followed by the upper dermis; antigen was also seen peripheral to the sweat gland and the hair follicle. In a few, antigen was found to be present in the deep dermis.

## 4. Discussion

The present study deals with two clinical categories of leprosy namely, 'early leprosy' and 'suspect leprosy, in the context of histological diagnosis. The term 'early leprosy' encompasses more than one type of leprosy and is potentially applicable to all early forms irrespective of type. For example, in the Ridley-Jopling classification of leprosy, early leprosy could be early TT[6].
Indeterminate leprosy, which has had a long classification history, was presently diagnosed using defined clinical criteria. Sensory deficit, though not always present in Indeterminate leprosy, was used a clinical criterion for the diagnosis of Indeterminate leprosy for several reasons.
The definitive diagnosis of Indeterminate leprosy requires the presence of an anesthetic skin lesion, or the presence of AFB or the presence of dermal nerve infiltration. However, in the study methodology. at the initial stage of clinical selection of cases, the additional corroborative

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criteria (the presence of Acid Fast Bacteria or dermal nerve infiltration) are not available[7]. With the availability of these additional criteria the original clinical classification could well change. This is illustrated in the clinical category of suspect leprosy where sensory loss was not present, but subsequent histopathological examination showed the features of Indeterminate leprosy in 2 cases, and features of BT leprosy in 7 cases.

In addition to the above reason for using sensory deficit as a criterion for the diagnosis of Indeterminate leprosy, the feature of sensory deficit also served to discriminate Indeterminate leprosy from the Clinically Suspect group of patients chosen for the study, which is otherwise quite similar to Indeterminate leprosy in visual appearance. Lastly, the Consensus Classification of Leprosy approved by the Indian Association of Leprologists states sensory impairment to touch and/or pain as a clinical characteristic of Indeterminate leprosy[8].

The host immune response in Indeterminate leprosy is yet to decisively develop, and, the disease my abort or progress to one of the immunologically defined types. Early TT/BT is immunologically. a step ahead in the evolution of the host tissue response, but like Indeterminate leprosy can resolve on its own without therapy. The other commonality shared between Indeterminate leprosy and early TT/BT is the underlying histological picture, which is often non-specific, due to which histological confirmation of the clinical diagnosis is often not Ponsible These commonalities formed the basis of selecting Idt leprosy[9].

Examination of the clinical data shows the age distribution and the symptom duration to be wide, which is in keeping with the observations of other workers and, descriptions in texts. The wide variation in symptom duration (1 month 60 months) is only to be expected as the progression of the early disease is both slow and unpredictable. Patients belonging to the paediatric age group were a small fraction (20%), a finding also supported by similar observations in other studies and in contrast to the earlier conviction that early leprosy (particularly the Idt form) was a disease of children. The predominance of males and the favoured distribution of the lesions in the extremities are also well recognized features of the early forms. The selected patients therefore represented a fairly characteristic group of early leprosy patients, notwithstanding the inherent institutional selection biases[10].

Immunostaining performed on tissue sections which were non-confirmatory from both the clinical categories of early leprosy and suspect leprosy, showed the presence of mycobacterial antigens in (34.37%) cases of early leprosy, and, in (17.14%) cases of suspect leprosy. Within the subcategories of early leprosy, antigen positivity in Indeterminate leprosy and early BT leprosy were similar (33.33% for Idt and 34.78% for early TT/BT). As the associated pathology seen in these cases is in all probability related to, if not consequent to, the antigen nearby, the histological diagnosis of leprosy can be considered confirmed. Similar antigenic presence was not seen in sections of normal skin which were simultaneously immunostained.

## 5. Conclusion

The present study, 100 cases of clinically diagnosed early leprosy (Idt leprosy, early TT/BT) and, 90 cases of clinically suspect leprosy were studied from the viewpoint of histological diagnosis. Homogeneity within the two groups was achieved by selecting only untreated cases and by defining the features required to make the clinical diagnosis. Biopsies were taken from the lesion peripheries, fixed in 10% buffered formalin, and, processed for routine histopathological

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examination. The histological diagnosis of leprosy, using specific defined criteria was made in (36%) in the clinical category of early leprosy, and in (24.44%) in the clinical category of suspect leprosy. These findings are consistent with the low (and variable) degree of histological confirmation reported in several studies in the chosen categories of cases.
Non-confirmatory sections of both the categories were then immunostained for the presence of mycobacterial antigen using the Indirect Immunoperoxidase technique. Antigen presence was seen in (34.37%) cases in the clinical category of early leprosy, and in (17.14%) cases in the clinically suspect group. The technically non-demanding, highly sensitive and inexpensive immunostaining procedure can be routinely used to augment the histological.

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