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Skin Biopsy in Leprosy

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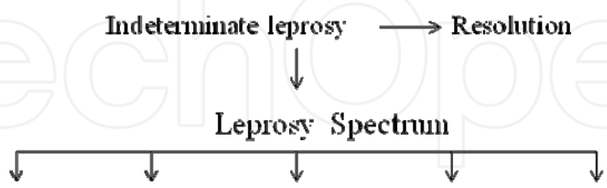
1. Introduction

Leprosy is a chronic infectious disease of varying severity caused by *Mycobacterium leprae* (*M. leprae*) a slowly multiplying pathogen. It is primarily a surface disease with lesions mainly involving the skin and peripheral nerves. Rarely visceral organs like liver, lymphnodes, bone marrow, eyes, bones and testes may be involved. Despite the prevalence rate of leprosy having steadily fallen throughout the world, it continues to be a cause of significant public health problem and morbidity in endemic regions. The disease is endemic in many tropical and subtropical countries but is declining in incidence. Globally, 211903 new leprosy cases were detected in 2010 (WHO, 2010). The most affected countries are India and Brazil with some countries in Sub Saharan Africa and Southeast Asia (Noordeen, 1995). The mode of transmission is still unknown but it is believed to be through inhalation of bacilli that are excreted from the nasal passages of the multibacillary patient. Direct person-to-person transmission through skin contact occurs rarely though there have been isolated reports of its transmission from hypodermic needles during skin tattooing or by physical trauma to skin.

2. Clinical and immunopathologic spectrum of leprosy

The sequence of disease pathogenesis in leprosy is complex and depends on the host immunological responses. *M. leprae* is non-toxic and the clinicopathologic manifestations are the result of this host-parasite interaction. (Ridley & Jopling, 1966; Ridley,1974). Leprosy is the classical example of the disease with an immunopathologic spectrum wherein the host immune reaction to the infective agent ranges from none to marked with a consequent range of clinicopathologic manifestations. Tuberculoid leprosy (TT) shows a high cellular response characterized by T-cell and macrophage activation and very few bacilli in the tissues. Lepromatous leprosy (LL) on the opposite pole shows an absent cellular immune response to *M. leprae* antigens with no macrophage activation and abundant bacilli in the tissues. The immunopathologic spectrum is a dynamic continuum in which the patients move in either direction according to the host immune response and treatment. The standard delineation follows the classification of Ridley and Jopling with categories defined along this spectrum by a combination of clinical, microbiological and histopathological indices: TT (tuberculoid), BT (borderline tuberculoid), BB (midborderline), BL (borderline lepromatous) and LL

(lepromatous leprosy). The TT and LL group of patients are stable, the former often self-healing and the latter remaining heavily infected unless given chemotherapy. The central point of the spectrum BB is most unstable with patients quickly downgrading to LL if not treated. Apart from these there are some patients who are labeled as 'indeterminate' leprosy and these are the patients with the earliest identifiable skin lesions that cannot be categorized definitely in the immunopathologic spectrum (Table1).



Features	TT	BT	BB	BL	LL
Number of Lesions	single	1- few	several	many	many
AFB	±	+	++/+++	+++/++++	> ++++
CMI	+++	+/++	±	_	_
Antibodies	+/±	+	++	+++	+++
Histopathology					
Epitheloid cells	++	++	+	_	_
Macrophages	_	_	+	++/+++	>+++
Lymphocytes	+++	+++	+	+	±

Table 1. Classical features of the leprosy spectrum

3. Leprosy reactions

Leprosy reactions are periodic episodes of acute inflammation caused by immune responses to *M. leprae* or its antigens superimposed on the chronic course of the disease. There are two main types of leprosy reactions depending on the underlying immunological mechanisms.

3.1 Reversal reaction

It is also referred to as type 1 reaction or upgrading reaction and occurs either spontaneously or after a course of chemotherapy and is due to increase in the delayed hypersensitivity reaction. There is influx of lymphocytes that are mainly of CD4+ subtype, especially of the Th1 class (Yamamura et al., 1991; Sreenivasan et al, 1998). During type1 reaction, increases in gene expression of several proinflammatory cytokines have been documented (Scollard et al, 2006). These episodes show cardinal features of inflammation such as erythema, edema, local warmth and tenderness over the skin lesions. The most important effect of a reversal reaction is not the skin but the peripheral nerves which show acute neuritis due to infiltration of lymphocytes and increased intraneural pressure that may result in nerve abscess, necrosis and loss of nerve function.

3.2 Erythema nodosum leprosum (ENL)

It is also known as type 2 reaction and occurs as an acute episode. LL leprosy patients with high bacillary index are more prone to develop ENL as compared to BL leprosy (Pfaltzgraaf et al, 1994). Based primarily on histopathological evidence, it had been proposed that ENL represents an Arthus-like phenomenon mediated by immune complexes (Wemambu et al, 1969). Immunoglobulins and complement deposits have been demonstrated in the skin lesions consistent with this hypothesis. However, neither circulating nor fixed immune complexes have been reproducibly demonstrated in ENL lesions. Other studies have demonstrated evidence of increases in circulating IFN-γ, TNF-α and IL12 and chemokines associated with chemotaxis of neutrophils. Increases in m RNA expression of these cytokines have also been observed in biopsies of skin lesions suggesting that cellular immune activation occurs locally (Sreenivasan et al, 1998; Scollard et al, 2006). Both immune complex deposition and enhanced T-cell reactivity have been demonstrated in peripheral blood and skin lesions. Clinically the skin shows redness, edema, tender plaques and nodules and rarely vesicle formation and ulceration. The eruptions are widespread and associated with constitutional symptoms like fever, arthralgia and involvement of other visceral organs.

3.3 Lucio phenomenon

It occurs in diffuse lepromatous (Lucio) leprosy patients who have either received no treatment or incomplete treatment and have been predominantly observed in Mexican and South American populations. In contrast to ENL, fever, tenderness and leucocytosis are absent. The lesions present as hemorrhagic, sharply marginated irregular crusted plaques that have tendency to ulcerate.

4. Skin biopsy technique

Skin biopsy is a key clinical procedure that is routinely required for the histopathological diagnosis of leprosy and is of paramount importance for correct histopathological classification, bacillary index, and follow-up of treatment response and disease activity. It is also useful for differentiating a relapse from a reversal reaction and to categorize reactions into type 1 or type2.

4.1 Principles of skin biopsy

Before planning a skin biopsy the patients should be informed about the procedure and its side effects. In some countries it is mandatory to obtain a signed consent form. If the patient is illiterate as is common in many endemic countries, care should be taken to explain the procedure verbally and obtain if required a thumbprint on the consent form. Institutional ethical committee's permission is not mandatory for diagnostic biopsies, however whenever the skin biopsy forms part of research project, it is essential to obtain a prior approval from such a committee. Any history of a bleeding disorder or intake of drugs that may interfere with hemostasis should be investigated prior to taking the biopsy.

4.2 Site selection

Deciding the site from where the biopsy will be taken is crucial. Generally the lesions with more advanced inflammatory changes should be chosen. The area from where the biopsy is to be taken should be marked with a skin marker by the physician (Figure 1A).The manifestations of leprosy lesions may vary but generally the site should be active and

representative. If a small hypopigmented patch suspected to be indeterminate leprosy is seen, the biopsy should be taken from the center of the lesion where the disease is active. If the lesion is an annular macule then the active spreading edge should be the ideal site of biopsy. If the patient has multiple lesions of different morphology, then more than one biopsy should generally be taken. Even though punch biopsies cause very little scarring care should be taken to avoid wherever possible the cosmetic sites such as face and areas with poor healing characteristics (Alguire & Mathes, 1998).The depth of the biopsy in leprosy should include full depth of the dermis and should extend till the subcutis so as to involve the deeper nerve bundles as well as subcutaneous fat.

4.3 Site preparation

Any common skin antiseptic such as isopropyl alcohol, povidone-iodine solution or chlorohexidine gluconate can be used to prepare the biopsy site (Pariser, 1989). The most commonly used anesthetic agent is 2% lignocaine 1ml of which is injected intradermally in stages while the needle is being inserted in the skin (Figure 1B). Since lignocaine is a vasodilator, small quantity of epinephrine may be added to decrease bleeding and prolong anesthesia. For small lesions the anesthetic can be directly injected into or adjacent to the lesion. For larger lesions a field block is recommended by placing a ring of anesthesia around the intended surgical site (Harrison, 1980).

4.4 Type of skin biopsy

Skin biopsy is a relatively simple procedure that is performed as an outpatient procedure and is essentially of three types

a. Incisional biopsy: is about 12 mm long is in practice, ideal for detection of early lesions. They are reserved for lesions that cannot be removed with a punch owing to their size, depth or location. These need sufficient expertise and invariably require sutures. They are usually reserved for deep inflammatory lesions or lesions involving the panniculus, ex. ENL. After taking the biopsy, the skin tissue is immediately placed in appropriate preservative depending upon type of investigation to be carried out.
b. Punch biopsy: is the most commonly performed skin biopsy and is the biopsy of choice for diagnostic purpose. Biopsy punch is a metal cylinder of variable diameter with a sharp cutting edge and usually attached to a plastic handle (Figure 1C). The diameter ranges from 3-10 mm. Ideally, 4-6mm punches are adequate but 3 mm is the minimum tissue that is considered sufficient for giving a consistently accurate histological diagnosis (Todd, et al., 1996). Biopsy punches blunt easily therefore disposable punches are recommended. Punch biopsies heal by secondary intention though lesions greater than 3mm can sometimes give unacceptable scarring and require 1-2 sutures. The skill required for such biopsies is easily mastered. In comparison to shave biopsies, punch biopsies have a low incidence of bleeding, infection or undesirable scarring (Grekin, 1989). After selecting the punch size, the skin is stabilized with thumb and index finger and slightly stretched perpendicular to the skin tension lines. After placing the punch perpendicular to the skin, a firm downward pressure is applied (Figure 1D). When the punch touches the subcutaneous fat there is a definite 'give' indicating that a full thickness cut has been made. The punch is withdrawn and the cylindrical piece of tissue is gently supported with blunt forceps or a needle tip. The tissue is immediately transferred into a fixative solution and a firm pressure is applied on the wound to prevent bleeding.

c. Shave biopsy: This type of skin sampling is quick, requires little training and sutures for closure are not required. They are usually meant for superficial lesions where the pathology is confined to epidermis and is not of use in leprosy.

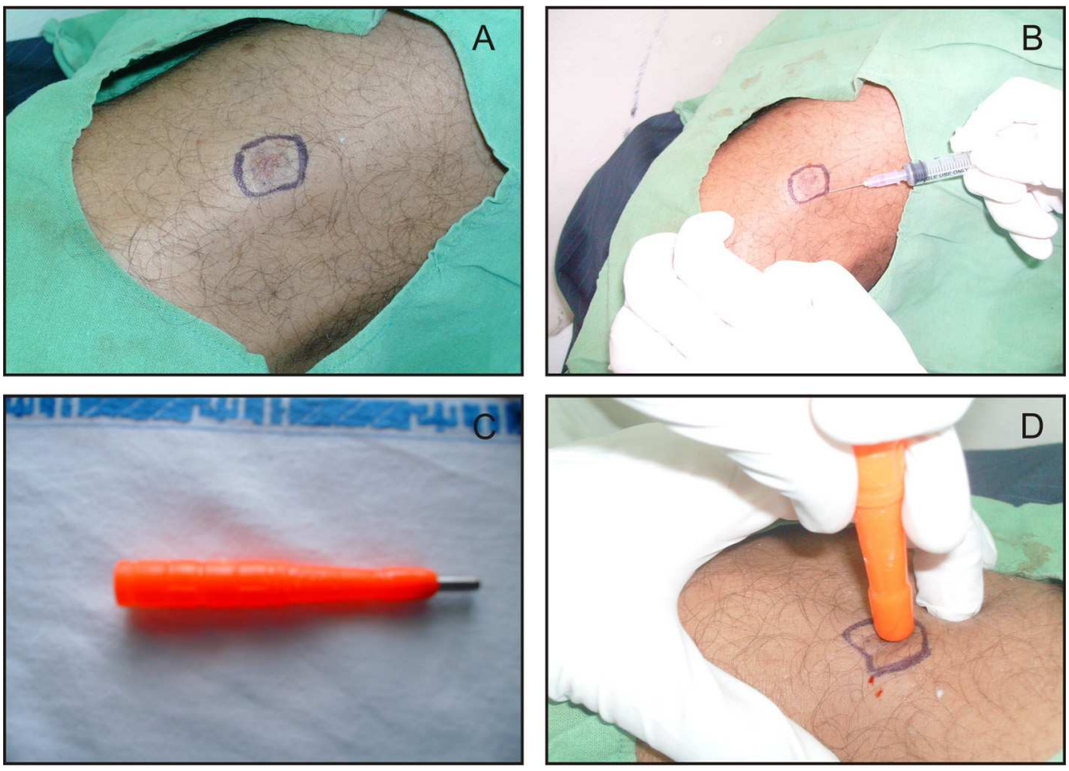


Fig. 1. Selected site for punch biopsy (A), intradermal injection of lignocaine (B), disposable needle punch (C), punch held perpendicular to the skin (D)

4.5 Wound closure

The simplest technique of wound closure is the simple interrupted suture. Primary closure of a punch wound can be accomplished with 1-2 single-layer interrupted sutures. Excisions can be closed in 1-2 layers. The qualities that are most important in suture selection are flexibility, strength, secured knotting and the infection potential (Moy, 1992). There are two broad varieties of sutures absorbable and non-absorbable. Absorbable sutures are made from mammalian-derived collagen (gut) and synthetic absorbable material such as polyglactic acid (vicryl), polyglycolic acid (dexon), poliglecaprone (monocryl) and polydioxanone (PDS). Absorbable sutures have a tensile strength of usually1-2 weeks and are usually placed deep in the wound to reduce skin tension for final closure. Non-absorbable sutures include silk, ethilon (nylon), polypropylene (prolene) and polybutester (dacron). Silk and nylon sutures cause considerable tissue inflammation and have now been largely replaced by synthetic sutures. The latter have a tensile strength ranging from 3 months to 2 years. All biopsy wounds can be dressed with a thin film of a broad spectrum antibiotic ointment and covered with a non adherent dressing topped with gauze dressing and tape (Telfer, 1993). Though skin biopsy is a very safe procedure rarely complications like bleeding, hematoma formation, allergic reaction and infection may still occur.

4.6 Preservation of the skin biopsy

As soon as the biopsy is taken it should be transferred to a preservative to prevent tissue autolysis and drying artifacts. The ideal fixative for standard paraffin embedded histopathological processing is 10% buffered formalin. This fixative is less damaging to the granuloma and works well for preserving the morphology of the AFB. Another fixative frequently used in the past was Lowy's fixative or the FMA (formaldehyde, mercuric chloride, glacial acetic acid) fixative. If properly undertaken FMA fixative gives good cellular details. The biopsy specimen should be completely immersed in the fixative that should be at least 10 times the volume of the skin biopsy. The same fixative can be used for subsequent staining for histochemical characterization and detection of phenolic glycolipid-1 (PGL-1) or lipoarabinomannan (Weng, 2000 ; Verhagen, 1999). For electron microscopy the fixative of choice is 2% glutaraldehyde. If direct fluorescence is to be done within 24 hours then skin biopsy should be thoroughly rinsed with normal saline and preserved at –20 ^{0} C. If the biopsy is to be used later, then Michel's liquid fixative is the recommended transport medium. This fixative is a proteolytic enzyme inhibitor and is reconstituted by N-ethylmalleimide, ammonium sulfate in a citrate buffer and will give the skin biopsy a shelf life of 4 weeks to 6 months. For molecular techniques like PCR and in-situ hybridization, buffered formalin fixed skin biopsies are considered suitable for PCR as other fixatives may inhibit the reaction (Singh et al, 2004). For RNA studies fresh skin biopsies are preserved immediately in a RNA stabilizing reagent (RNA later) and preserved at -80^{0} C.

5. Protocol for staining of Acid Fast Bacilli (AFB) in paraffin embedded tissue samples

5.1 Modified Fite - Faraco stain (Job & Chacko, 1986)

a. *Ziehl-Neelsens Carbol fuschin solution (200ml) are*
   Basic fuschin: 2 gm
   Melted-phenol: 10 ml
   Absolute alcohol: 20 ml
   Distilled water: 170 ml
b. *5% Sulfuric acid (200ml)*
   Concentrated sulfuric acid (1.0 N):10ml
   Distilled water: 190 ml
c. *Harris Hematoxylin*
   Hematoxylin: 5 gm
   Absolute alcohol: 50 ml
   Potassium alum: 100 gm
   Mercuric oxide: 2.5 gm
   Glacial acetic acid: 25 ml
   Distilled water: 1000 ml

5.2 Staining procedure

- Deparaffinize sections in a mixture of xylene and peanut oil (2 parts of xylene,1 part of peanut oil), two changes of 6 minute each
- Drain, wipe off the excess oil and blot with filter paper
- Wash in running tap water for 4 minutes
- Stain with Ziehl- Neelsen's carbol fuschin solution for 30 minutes at room temperature

- Wash in tap water for 2 minutes
- Differentiate sections in 5% sulfuric acid in 25% alcohol for two changes of 2 minutes each
- Wash in running tap water for 5 minutes
- Drain the excess water, blot dry the sections with a filter paper. Do not dehydrate in alcohol.
- Clear in two changes of xylene and mount

This staining method has two modifications from the standard Fite-Faraco stain for AFB. Firstly, use of alcohol is minimized to prevent excessive decolorization. Secondly, the counterstain is hematoxylin instead of methylene blue. AFB show red colour and nuclei stain dark blue giving the localization of AFB in relation to the cells.

6. Fluorescent microscopy in leprosy

Demonstration of *M. leprae* during histopathological examination of early lesions is an important criterion in the confirmation of leprosy diagnosis. The sensitivity of detection of AFB by histological means remains poor because about 1000 bacilli per cubic centimeter of tissue must be present in order to detect 1 AFB in a section. To enhance the detection rate it is recommended that atleast six sections be examined before declaring them negative. Routine acid fast stains are not so sensitive due to the variability in their ability to decolorize AFB using acid-alcohol. In the search for more sensitivity, a fluorescent method of staining similar to *M. tuberculosis* has been used and is reportedly 1.5 times more sensitive in demonstrating *M. leprae* than Fite-Faraco stain (Nayak, et al., 2003). Immunofluorescence (IF) is also useful in leprosy research where it is a valuable tool in studying localization of antigens like immunoglobulins, HLA-DR, cell surface markers and cytokines on frozen skin biopsies. IF is a better method for studying double labeling of antigens as compared to histochemical staining on formalin fixed paraffin embedded tissue.

7. Numerical indices in a skin biopsy

7.1 Granuloma Fraction (GF)

The fraction of the dermis in a section occupied by a granuloma is observed visually under a low-power objective (x 4) and expressed as fractions. For e.g. 1.0 indicates that the whole of the dermis is occupied by the granuloma, 0.2 that one-fifth is occupied. The range from 1.0-0.1 can be estimated quite accurately if the biopsy specimen extends down up to the subcutis. Being an arithmetic index, GF is sensitive and can adequately reflect the degree of skin infiltration.

7.2 Bacterial Index (BI)

BI is the universally accepted method of assessing the load of acid fast bacilli in a leprosy patient defined by Ridley (Ridley, 1958).The samples tested can be the skin biopsy or slit smears of lesional sites. The latter is obtained as serous fluid by a superficial slit with a sterile blade such that no blood contaminates the fluid. The fluid at the tip of the blade is smeared on to the glass slides which are then stained with Fite-Faraco stain. 3-6 sites from the body are recommended for testing. Slit smear examination is undertaken periodically for assessing improvement with drug therapy and is less invasive than the skin biopsy and is useful in the field sites where facilities for histopathology may not be available.

The bacterial load in skin biopy or slit skin smears is graded using an oil immersion objective as:

0:	No bacilli found in 100 microscopic fields
1+: 1-10	bacilli in 100 microscopic fields
2+: 1-10	bacilli in 10 microscopic fields
3+: 1-10	in one microscopic field
4+: 10-100	in one microscopic field
5+: 100-1000	bacilli in one microscopic field
6+: Over 1000	bacilli and globi in one microscopic field

The BI is calculated as mean BI of the sites tested.

7.3 Histopathological Index (HI)

HI is the logarithmic index of bacilli in a biopsy and makes use of the GF and BI to assess the number of bacilli in the tissue section. The actual number of bacilli in the volume of given tissue can be calculated from the HI

8. Histopathology of leprosy

Taking a skin biopsy from the advancing cutaneous lesion in a patient suffering from leprosy is the single most informative procedure for diagnosis of leprosy spectrum and because of its ability to provide insight into the underlying disease process. The skin biopsy captures the pathology of the lesion at a given point and is a very useful resource that can be used for further investigation of the patient (Figure 2A-F). Standard histopathological examination of the formalin-fixed paraffin embedded skin tissue can provide information regarding cellular morphology, presence of AFB and can be further enhanced by techniques like imunohistochemistry, immunoelectron microscopy and molecular studies. Pure neuritic leprosy is another variant which shows typical tuberculoid or borderline features in nerves. Nerve biopsy is required for diagnosis in such cases but if the subject also shows skin lesions then nerve biopsy is not considered to be essential.

There are several useful applications of histopathological examination of the skin biopsy from a leprosy patient namely:

a. to confirm the diagnosis of leprosy
b. to accurately classify the lesion in the leprosy spectrum
c. for identification of the bacillary load in the tissue
d. in assessment of disease activity and response to treatment
e. for the diagnosis of a leprosy reaction

8.1 Indeterminate leprosy

Indeterminate leprosy is the earliest detectable skin lesion comprising one or few hypopigmented macules with no clear sensory changes. The skin biopsy may show mild accumulation of lymphocytes and macrophages and an occasional AFB either in the non-inflammed nerve, erector pili or in the subepidermal zone in the very early stages. It may show neuritis evidenced by Schwann cell proliferation and infiltration of the nerve fibres with lymphocytes. Nerve infiltration is the most significant feature of leprosy when the rest of the skin shows non specific changes. Moreover, the histological changes are known to

precede the clinical manifestations by atleast 3 months (Ridley, 1978). Most indeterminate leprosy cases are known to heal spontaneously (Crawford, et al., 1977) but since it is not possible to predict which indeterminate cases will evolve into full blown leprosy, it is considered ethical to treat all the patients.

8.2 Tuberculoid Leprosy (TT)

Primary polar tuberculoid leprosy has large and compact epitheloid cell granulomas along the neurovascular bundles with lymphocytes. Langhans giant cells are typically scanty or absent and AFB are rare to find. Epitheloid cell granuolmas always erode into the basal layer of the epidermis. The dermal nerves may be either obiliterated and completely effaced or eroded by the dense lymphocyte cuff.

8.3 Borderline Tuberculoid leprosy (BT)

The epitheloid granulomas of BT do not invade into the epidermis and have less lymphocytes in comparison to TT. The granulomas are arranged in a curvilinear pattern along the neurovascular bundle. Nerve erosion by the granuloma is typical and AFB are scanty (ranging from BI 0-2) and are more readily detected in the Schawann cells of the nerves. In addition to nerves, the granuloma can also involve the sweat glands and the erector pili muscle.

8.4 Midborderlne leprosy (BB)

The histopathology in BB shows almost equal admixture of epitheloid cells and macrophages without forming a distinct granuloma. The lymphocytes are scant and scattered and multinucleate giant cells are absent, a feature that helps it to be distinguished from BT. AFB may be frequent (ranging from BI 2-4)

8.5 Borderline Lepromatous leprosy (BL)

The predominant cells in the granulomas are macrophages with occasional epitheloid cells arranged in patches. Lymphocytes are sparse, AFB are abundant (ranging from BI 3-5) but usually not present as globi. Perineural fibroblast proliferation forming an 'onion skin' in cross section is a typical feature. Early evidence of foamy change may be detectable.

8.6 Lepromatous Leprosy (LL)

The typical features consist of a flattened epidermis separated from the dermal infiltrate by a dense zone of normal collagen. The macrophage granuloma of LL is large and expansile consisting of sheets of foam cells with rare presence of lymphocytes. The foam cells harbor abundant AFB (ranging from BI 4-6). The solid bacilli are stacked like cigars and appear as globi. Such appearance is the rule rather than an exception. In contrast to tuberculoid leprosy, the nerves in the skin of LL patients may contain considerable AFB, however, the morphological features of the nerve is fairly well preserved in the earlier phase of the disease before eventually becoming fibrotic.

8.7 Lucio leprosy

The histopathology of this Mexican variant is similar to LL but with a characteristic heavy bacillation of the small blood vessels of the skin leading to thrombosis of vessels and ischemia and ulceration called as the 'lucio' phenomenon.

8.8 Histiod leprosy

This is another variant of LL, which shows the highest load (BI 6) of solid staining AFB arranged in clumps and sheaves. The macrophage reaction is unusual in the sense that the macrophages become spindle shaped and oriented in a storiform pattern reminiscent of a fibrohistiocytoma.

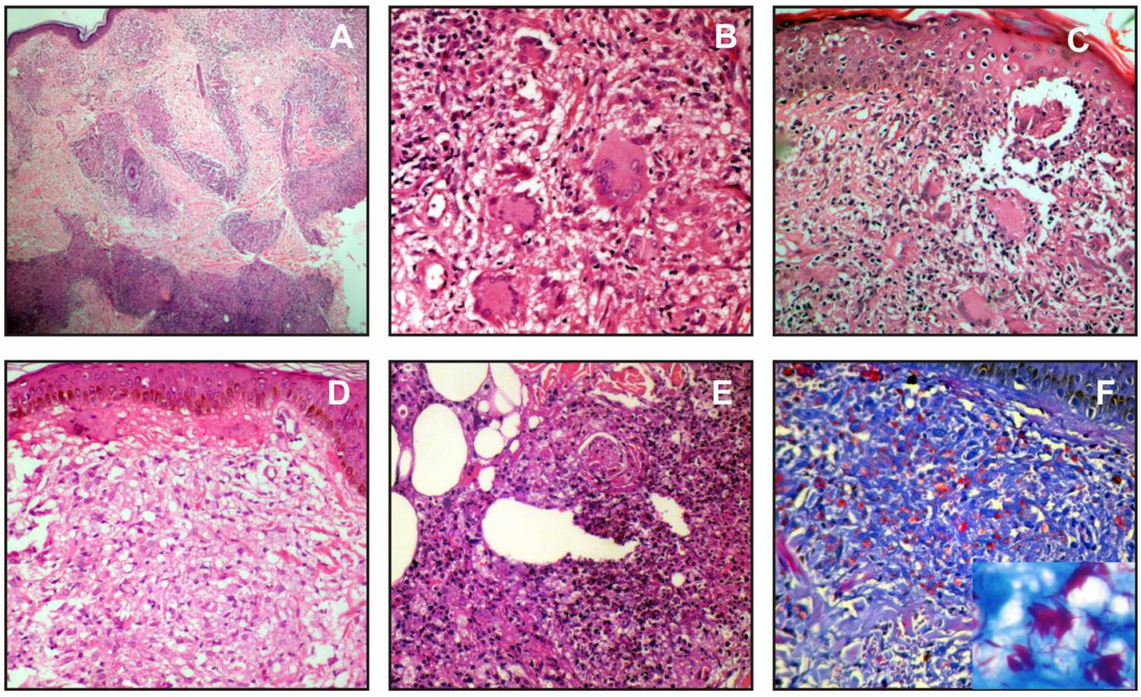


Fig. 2. Photomicrograph showing compact epithelioid granulomas in BT(A), multinucleate Langhans giant cells (B), epidermal erosion in leprosy reversal reaction (C), foamy macrophage granuloma of LL (D), ENL showing infiltration in subcutis along with neutrophils (E), Fite stain showing numerous globi (F) and AFB (inset) (magnification 40X except for inset which is 100X oil immersion)

9. Histopathological differential diagnosis

Tuberculoid leprosy needs to be differentiated from other granulomatous dermatitides. Cutaneous tuberculosis is the most important differential diagnosis which has to be excluded. The epidermis in tuberculoid leprosy is usually flat and not hyperplastic as in tuberculosis. The arrangement of the granulomas in leprosy is along the neurovascular bundles giving an oblong pattern to the granuloma unlike tuberculosis where there is intense and sometimes lichenoid pattern of the chronic granulomatous infiltrate. The dermal nerve twigs are spared by the infiltrate in tuberculosis. The presence of granuloma or AFB in the nerve is a conclusive proof of leprosy. Cutaneous sarcoidosis may sometimes be confused with tuberculoid leprosy as fibrinoid necrosis may be found in both these entities. The granuloma of sarcoidosis show paucity of lymphocytes and are more confluent and show fibrosis around the granuloma. Other granulomatous lesions like leishmaniasis or granulomatous post kala azar dermal leishmaniasis also need to be excluded by demonstration of Leishman-Donovan bodies and frequent presence of plasma cells. Borderline lepromatous and pure lepromatous leprosy may

be confused with histocyte-rich lesions like xanthomas, however demonstration of AFB in these lesions usually solves the diagnostic dilemma.

10. Histopathology of leprosy reactions

Histopathological examination of a skin biopsy of reversal reaction will show dermal and intragranuloma edema. The granulomas become more epitheloid, show infiltration of lymphocytes within and around them and the Langhans giant cells become increased in number and bigger in size and may also show bizarre shapes. The granulomas also erode into the epidermis representing the upgrading reaction. In addition caseous necrosis may be evident in the nerves. At the molecular level, the events in type 1 reaction are responses to an array of cytokines secreted by the lymphocytes and macrophages. The skin and nerves are infiltrated by interferon-γ and tumor necrosis factor-α secreting CD4+ lymphocytes and are responsible for the inflammation and tissue damage.

On histopathology, a type 2 reaction is characterized by varying degree of polymorphonuclear infiltration superimposed on already existing lepromatous granuloma. The influx of neutrophils can be intense so as to form neutrophilic microabscess. Edema is frequently present in the dermis. Deposition of immune complexes in the small cutaneous capillaries, arterioles and venules result in necrotizing vasculitis. The downgrading reaction is reflected by deeper infiltration of foamy histiocytes into the subcutaneous fat. The AFB are fragmented and granular. Superficial ulceration, bulla formation and necrosis may sometimes supervene.

11. Skin biopsy in relapse

Relapse in paucibacillary (PB) leprosy may be defined as appearance of a new skin lesion or increase in the size of a pre-existing skin lesion provided there is a strong clinical and/or histopathological evidence of leprosy in such a lesion (Boeriggter, 1991). The difficulty in relapse in PB is to differentiate relapse from a type 1 reaction or drug resisitance. A true relapse can be detected histopathologically only after recording complete histological resolution of the earlier lesion in a previous biopsy. A relapse indicates that the AFB have survived despite antileprosy therapy and have multiplied and released antigens to produce fresh granulomas at the site of original lesion and show solid staining viable AFB. Alternatively, a fresh infection or reinfection may have occurred on reexposure. The cause of relapse may be irregular, inadequate therapy, presence of high initial BI, presence of 'persistors' bacilli that had remained dormant. Relapse in multibacillary (MB) leprosy are characterized in the early stage of relapse by emergence of foci of spindle shaped macrophages with granular, eosinophilic cytoplasm along with small foci of persisting foamy histiocytes and solid staining AFB in these patients who have not completely become smear negative. In late stage of relapse perineural thickening, fibrosis along with Schwann cell and endothelial cells packed with solid AFB are detectable.

A relapse can be differentiated from a reversal reaction by the fact that reversal reaction usually occurs within 6 months of release from treatment while relapse occurs after a year of release. The reversal reaction additionally shows erythema, edema, neuritis and fewer new lesions unlike relapse. Reactions respond well to steroids and show complete subsidence of lesions in 2-4 weeks whereas the relapse will show only partial or no response. Owing to chronic course and long duration of treatment, drug resistance is an emerging problem in leprosy. Drug resistance can be primary or secondary due to mutant bacilli surviving in a

setting of monotherapy as in dapsone resistance or irregular therapy as seen in rifampicin resistance. While there is appearance of new lesions and patient downgrades in drug resistance, in relapse there is reappearance over the old lesions but the patient rarely downgrades.

12. Use of molecular tools in leprosy

Definitive identification of *M. leprae* is somewhat problematic since the organism is not cultivable. This problem is further confounded by increased prevalence of other mycobacterial infections of skin. Molecular tests like real time PCR are being used for the rapid detection and quantification of bacterial DNA content from the clinical biopsy samples in which AFB were not detectable by conventional histopathological staining (Martinez et al., 2006). Molecular methods can help in rapid direct identification of drug resistance. Mutations in the *M. leprae* genome that are associated with resistance to several drugs have been identified and DNA analysis to detect these mutations has largely replaced the mouse footpad technique. Mutations in targeted genes can be identified by molecular approaches such as probe assays and sequencing and the magnitude of drug resistant mutants can be assessed from the biopsy samples (Honore & Cole, 2001)). Decrease in PCR signals have been shown to correlate with effect of therapy and cases showing persistence of signals for longer periods have been shown to correlate with higher relapses. (Wood & Cole, 1989; Gupta et al, 2001; Singh et al,1999). These molecular tools therefore can be useful adjuncts to clinical and histopathologic diagnosis of leprosy.

Molecular tools are also being increasingly used for research. Pathogenesis of leprosy is being explored by studying the expression of cell markers, cytokines and growth factors. *M. leprae* being noncultivable by conventional means constrained information on its metabolism and strain variations. Subsequent to the sequencing of its genome from an Indian strain, it has become possible to understand its genetic makeup. In addition, it has become possible to characterize strains of *M. leprae* using short tandem repeats of nucleotides in the genome sequence of the bacillus. Global studies are underway to study the distribution of strains as well as pathogen transmission by DNA based signatures of the bacilli. Since *M. leprae* is noncultivable, such strains are obtained from skin biopsies. DNA is extracted and amplified by PCR and probed with appropriate probes. Using archival material it has been possible to investigate the evolution of *M. leprae* as well as its origin in man in Africa and its subsequent spread to other parts of the world along with its human host (Monot et al, 2009; Singh & Cole, 2011). Recent evidence indicates that the organism from disseminated lepromatous leprosy endemic to Mexico and Latin America has different molecular signatures to *M. leprae* and has thus been given the new nomenclature of *M. lepromatosis* (Han et al, 2009).

13. Conclusion

Skin biopsy is integral to the understanding of leprosy, its causative organism and for monitoring its relapse and drug resistance. Diagnosis of leprosy has been based on classical cardinal signs, characteristic histopathological findings and demonstration of acid-fast bacilli both from the skin smears and skin biopsies of these lesions. The current primary goal is early diagnosis of this disease in order to interrupt the transmission by early treatment. Histopatholgical examination of the lesional skin is still the gold standard in confirmation of its diagnosis and its classification based on the immunopatholgic status. As new serological

and molecular tests become available for the early diagnosis of leprosy, its reactions and resistance to drugs, skin biopsies will continue to compliment these emerging tools for providing more insights into the pathology of this fascinating disease.

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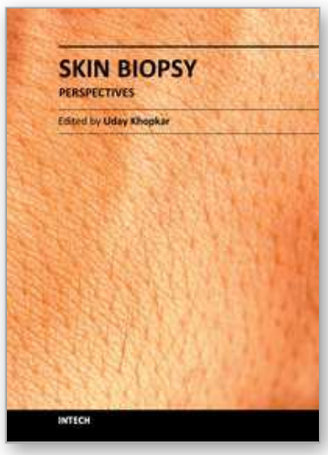
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Skin Biopsy - Perspectives is a comprehensive compilation of articles that relate to the technique and applications of skin biopsy in diagnosing skin diseases. While there have been numerous treatises to date on the interpretation or description of skin biopsy findings in various skin diseases, books dedicated entirely to perfecting the technique of skin biopsy have been few and far between. This book is an attempt to bridge this gap. Though the emphasis of this book is on use of this technique in skin diseases in humans, a few articles on skin biopsy in animals have been included to acquaint the reader to the interrelationship of various scientific disciplines. All aspects of the procedure of skin biopsy have been adequately dealt with so as to improve biopsy outcomes for patients, which is the ultimate goal of this work.

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