International Journal of Advanced Research in Biological Sciences
ISSN: 2348-8069
www.ijarbs.com
DOI: 10.22192/ijarbs Coden: IJARQG(USA) Volume 6, Issue 3 - 2019

Research Article

DOI: http://dx.doi.org/10.22192/ijarbs.2019.06.03.009

Study of Immunohistochemistry for mycobacterial antigen in skin biopsy for cases (IDT) and disease controls

Archana Singh Chauhan* and Tabrez Ahmad**

*Department of Zoology, National PG College, Lucknow
**Department of Zoology, BSNV PG College, Lucknow
E-mail: *arvindsingh.luck@gmail.com*

Abstract

Immunohistochemistry was done for mycobacterial antigen and mycobacteria detection in tissue sections using primary antibody [Rabbit antimycobacterium bovis (BCG)] in all cases and control. The staining was done in all tissue sections. Study group consisting of 75 indeterminate leprosy and 100 other leprosy groups of spectrum (disease controls) i.e. TT (n=20), BT (n=20), BB (n=20), BL (n=20) and LL (n=20). Negative tissue was stained for *M. bovis* in order to assess for expression in controls. Positive cases showed brown cytoplasmic staining as well as clusters of bacteria in heavily infected cases.
The intensity and extent of staining was marked in cases of multibacillary leprosy borderline lepromatous 20 of 20 (100%) cases and lepromatous 20 of 20 (100%) cases. Only 8 of 20 (40%) cases of borderline borderline leprosy showed positivity against mycobacterial antigen.

Keywords: Immunohistochemistry, Mycobacterium, antigen, leprosy

Introduction

Leprosy is probably the oldest known disease to mankind and is widely prevalent in India. According to the latest data presented by the National Leprosy Eradication Programme NLEP, the annual new case detection rate is 10.48 per 100,000 population, that is, about 1.27 lakh new cases in 2010-11 (NLEP,2011) Prevalence rates have gone up in some endemic areas and leprosy continues to remain a public health burden.

Histopathology is the key to diagnosis of leprosy. Diagnosis usually is not difficult in the lepromatous spectrum because of the abundance of bacilli in the lesions (Tirumalae *et.al*. 2014). In tuberculoid and indeterminate forms, diagnosis is more difficult. The granulomas overlap morphologically with other infectious and non-infectious lesions, notably tuberculosis, sarcoidosis and fungal infections, which are also common in our country (Thomas *et.al*, 1999). The quest for bacilli by Fite-Faraco technique is often unrewarding. It is cutaneous nerve involvement by the inflammatory reaction that permits the differential diagnosis of leprosy from the other cutaneous granulomas. Even this feature is not easily discernable always. The pathologist often faces difficulties in visualizing nerve impairment, especially in inadequate biopsies. In sections stained with H and E, nerve remnants within granulomas are confused with epithelioid cells, fragments of arrector pili muscles, and small vessels (Fleury and Bacchi, 1987).

Govindan *et.al* (2018) studied the immunohistochemistry analysis in delineating the complex immunological processes involved in leprosy and leprosy reactions.

Manandhar *et.al* (2013) studied the classification of leprosy on the basis of histopathological criteria and correlation with clinical information and bacteriological examination so as to facilitate accurate therapy to prevent undesirable complication

Indeterminate leprosy is a diagnostic problem both for the clinician and the pathologist. The clinical picture is vague and histopathological changes are non-specific. It is proposed to diagnose the patients with Ldt. Leprosy.

Materials and Methods

The study was carried out at the skin outpatients department (OPD) and immunology unit of Department of Pathology, K.G.'s Medical College, and Lucknow from October 1998 to Dec. 2001. The present study has been carried out on suspected cases of leprosy with doubtful patches. The cases presented at the outpatient department of skin (OPD), Department of Medicine, K.G.'s Medical College, Lucknow, INDIA. A written informed consent was taken from every patient who was enrolled in this study.

(a) Controls

Age and sex matched healthy controls has been studied. Patients of other types of leprosy were used as disease control. Present study were categorized under the following parts:

Part I: Clinical Assessment: In this part study of 75 indeterminate (Idt) leprosy cases and 100 other suspected leprosy subjects were studied.

Part II: Immunohistochemistry: Done for mycobacterial antigen and bacilli detection through indirect immunoperoxidase staining in tissue section (Mshana *et al*., 1982) with some modification.

Results and Discussion

Immunohistochemistry was done for mycobacterial antigen and mycobacteria detection in tissue sections using primary antibody [Rabbit antimycobacterium bovis (BCG)] in all cases and controls .The staining was done in all tissue sections by the procedure described earlier in material and method.

Study group consisting of 75 indeterminate leprosy and 100 other leprosy groups of spectrum (disease controls) i.e. TT (n=20), BT (n=20), BB (n=20), BL (n=20) and LL (n=20). Negative tissue was stained for M. bovis in order to assess for expression in controls. Positive cases showed brown cytoplasmic staining as well as clusters of bacteria in heavily infected cases.

Immunohistochemical detection of bacillary antigens were observed in 64 of 75 (83.3%) indeterminate cases, and seen more frequently in the cytoplasm of isolated cells of perivascular inflammatory infiltrate followed by the arrectores pilorum muscles. It was also detected in the periadenexial inflammatory infiltrate and at the endothelial lining of the small dermal vessels (Table 1).

The intensity and extent of staining was marked in cases of multibacillary leprosy borderline lepromatous 20 of 20 (100%) cases and lepromatous 20 of 20 (100%) cases. Only 8 of 20 (40%) cases of borderline borderline leprosy showed positivity against mycobacterial antigen.

Cases of paucibacillary leprosy including tuberculoid leprosy & borderline tuberculoid leprosy showed variation in intensity of staining. Few Schwann cells in nerve bundles also showed positive staining. Positivity in immunohistochemistry was observed in 15 of 20 (75%) tuberculoid cases and 10 of 20 (50%) borderline tuberculoid leprosy cases (Table 1& Fig 1). Negative control sections did not exhibit any antigen deposits.

Table 1: Immunohistochemistry for mycobacterial antigen and mycobacteria in skin biopsy of cases (ldt) and disease controls

Types of leprosy	Positive Number (%)		Total No. of Negative	
	Positive Number	%(age)	Negative Number	% (age)
Cases				
Indeterminate leprosy (ldt) (n=75)	64	85.3	11	14.6
Disease Controls (n=20)				
Tuberculoid leprosy (TL) (n=20)	15	75	5	25
Borderline tuberculoid (BT) (n=20)	10	50	10	50
Borderline borderline (BB) (n=20)	8	40	12	60
Borderline lepromatous (BL) (n=20)	20	100	-	-
Lepromatous leprosy (LL) (n=20)	20	100	-	-

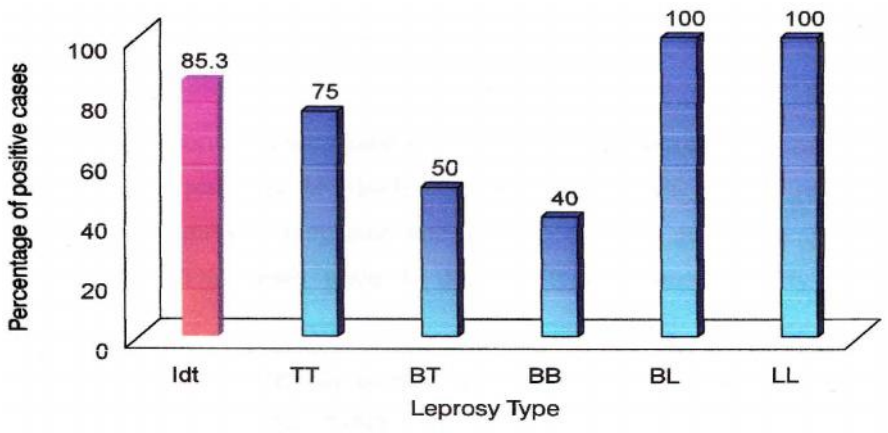

Fig: 1 Immunohistochemistry for mycobacterial antigen and mycobacteria in skin biopsy of cases (Idt) and disease controls

Dharmendera lepromin antigen skin test were done in all cases of indeterminate leprosy and disease control i.e., other leprosy groups of spectrum (Tuberculoid leprosy to lepromatous leprosy).Thee early (Fernandez reaction probably elicits pre-existing, delayed type hyper sensitivity (DTH), while the late (Mitsuda reaction) reflects cell mediated immunity (CMI).

Results of Mitsuda skin test revealed gradual increase in diameter of indurations from LL of TL patients. Similar results were obtained in early reaction.

Early response (delayed type hypersensitivity) was measured after 48 hours of intradermal injection only 28 of 75 (37.3%) indeterminate leprosy cases gave positive response in which 20 of 75 (26.7%) cases gave 1^+ response, 5 of 75 (6.7%) cases gave 2^+ response, 2 of 75 (2.7%) cases gave 3^+ response and only 1 of 75 (1.3%) patients gave 4^+ DTH response. The test was negative in 47 of 75 (62.7%) indeterminate cases (Table 2).

In disease control group nineteen of 20 (95%) tuberculoid leprosy cases had positive DTH response in which 15 of 20 (75%) cases gave 1^+ response, 2 of 20 (10%) cases gave 2^+ response, one of 20 (5%) cases gave 3^+ response and only one of 20 (5%) cases gave 4^+ delayed type hypersensitivity response (Table 2 & Fig 2).

Seventeen of 20 (85%) borderline tuberculoid showed positive DTH response in which 15 of 20 (75%) cases gave 1^+ response and 2 of 20 (10%) cases gave 2^+ responses (Table 2).

18 of 20 (90%) borderline leprosy cases gave only 1^+ DTH response (Table 23a).5 of 20 (25%) borderline lepromatous leprosy cases gave 1^+ delayed type hypersensitivity response and early reaction for lepromatous leprosy was negative in all 20 of 20 (100%) cases (Table 2 & Fig 2).

Table 2: Results of Dharmendera Lepromin Skin Test (delayed type hypersensitivity) after 48 hrs. in cases (Idt) and disease controls.

Leprosy types	DTH skin response							
	1^+	2^+	3^+	4^+	Total No. of positive		Total No. of Negative	
					No.	%	No.	%
Cases:								
Indeterminate leprosy (Idt) (n=75)	20	5	2	1	28	37.3	47	62.7
Disease controls:								
Tuberculoid leprosy (TL) (n=20)	15	2	1	1	19	95	1	5
Borderline tuberculoid (BT) (n=20)	15	2	-	-	17	85	3	15
Borderline borderline (BB) (n=20)	18	-	-	-	18	90	2	10
Borderline lepromatous (BL) (n=20)	5	-	-	-	5	25	15	75
Lepromatous leprosy (LL) (n=20)	-	-	-	-	-	-	20	100

Abbreviation

1^+ = induration between 6 to 10 mm diameter.
2^+ = induration between 11 to 15 mm diameter.
3^+ = induration between 16 to 20 mm diameter.
4^+ = induration between 21 to 25 mm diameter.
-ve = induration < 6 mm diameter.

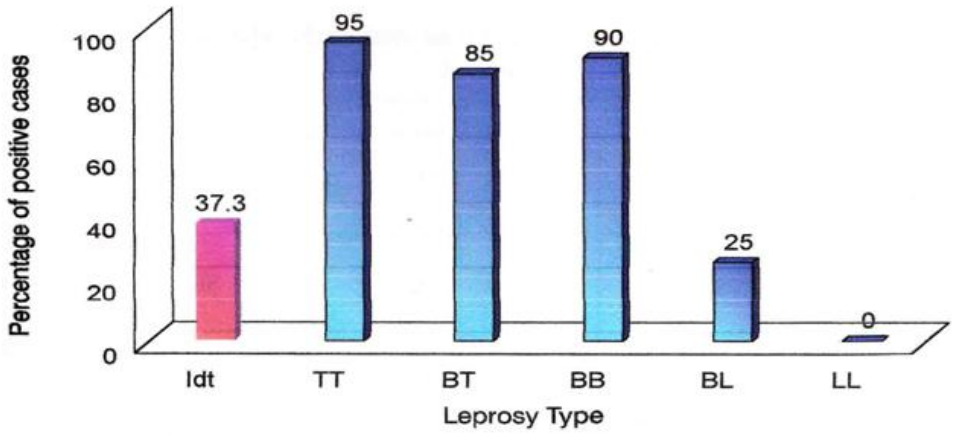

Fig: 2 Results of Dharmendera Lepromin skin test (delayed type hypersensitivity) after 48 hrs in cases(Idt) and disease controls

Late reaction (Mitsuda response) was measured after 3 weeks of intradermal injection. Only 23 to 75 (30%) indeterminate leprosy patients gave 1^+ mitsuda response (Table 3).

In disease control group eight of 20 (40%) tuberculoid leprosy case gave 1^+ mitsuda response, 6 of 20 (30%) borderline tuberculoid gave 1^+ mitsuda response 3 of 20 (15%) borderline borderline leprosy cases gave 1^+ mitsuda response and only one of 20 (5%) case of borderline lepromatous gave 1^+ positive response. Mitsuda response was negative in all 100% cases of lepromatous leprosy (Table 3& Fig 3).

Table 3: Mitsuda reaction in cases (Idt) and disease controls after 3 weeks

Leprosy types	DTH skin response							
	1^+	2^+	3^+	4^+	Total No. of positive		**Total No. of Negative**	
					No.	**%**	**No.**	**%**
Cases:								
Indeterminate leprosy (Idt) (n=75)	23	-	-	-	23	30	52	69
Disease controls:								
Tuberculoid leprosy (TL) (n=20)	8	-	-	-	8	40	12	60
Borderline tuberculoid (BT) (n=20)	6	-	-	-	6	30	14	70
Borderline borderline (BB) (n=20)	3	-	-	-	3	15	17	85
Borderline lepromatous (BL) (n=20)	1	-	-	-	1	5	19	95
Lepromatous leprosy (LL) (n=20)	-	-	-	-	-	-	20	100

Abbreviation

1^+ = induration between 5 to 8 mm diameter.
2^+ = induration between 9 to 12 mm diameter.
3^+ = induration between 13 to 16 mm diameter.
4^+ = induration between 17 to 20 mm diameter.
-ve = induration < 5 mm diameter.

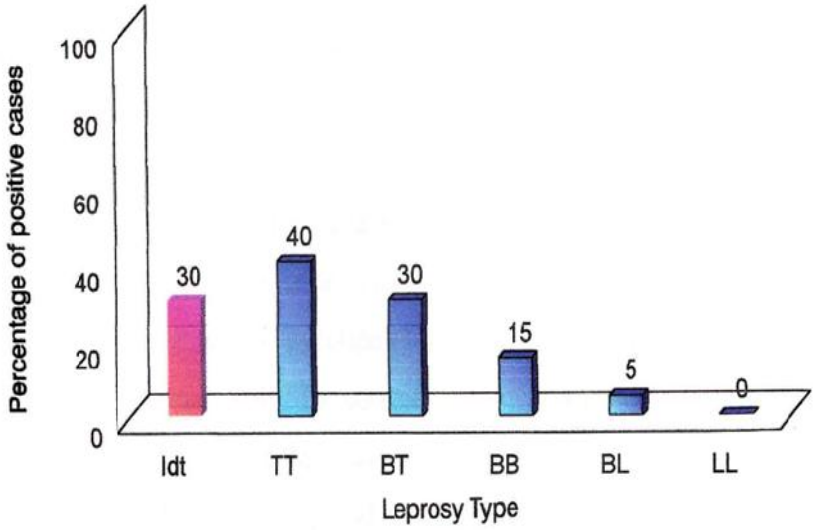

Fig: 3 Results of Mitsuda reaction in cases (Idt) and disease controls

Leprosy is a neurotropic disease caused by *Mycobacterium leprae*. This property is invaluable to a histopathologist making its microscopic diagnosis. In the lepromatous end of the spectrum, a confident diagnosis is possible owing to the abundance of acid fast bacilli, which may be detected even on H and E sections with a bluish hue (Rindley and Jopling, 1966 and Tirumalae *et.al*, 2014)

Indeterminate Leprosy (Idt) is considered to be the earliest manifestation of the disease. Indeterminate leprosy presents as single or multiple, asymmetrical, slightly hypopigmented (pale) or faintly erythematous and usually ill-defined (hazy) macules on the skin. Sensation on the affected area is normal or slightly impaired, while sweating and hair growth are usually unaffected. The peripheral nerves are normal. Slit-skin smears are mostly negative (Leiker *et al*, 1983). However, careful examinations of well-stained serial sections usually reveal acid-fast bacilli in dermal nerve fibrils infiltrated with lymphocytes (Browne, 1984). The lepromin test may be either negative or positive. Indeterminate leprosy is usually self-limiting or self-healing, but may progress to other forms of leprosy (i.e. tuberculoid, borderline tuberculoid, midborderline, borderline lepromatous or lepromatous leprosy) (Ackerman *et.al*. 2005).

Guha (1982) have reported in his study that 95% of individuals who come in contact with *M. leprae* do not develop an overt disease. It begins as an indeterminate form that may undergo spontaneous curve or may progress to different forms of leprosy (TT, BT, unstable form of BB, BL or LL). The clinical form of the disease correlates with the T-cell mediated immune response rather than to the direct damage caused by the bacilli (Takahashi, *et al*., 1992)

According to the WHO recommendation published in 1988, any case of paucibacillary leprosy (Idt, TT and BT) showing skin smear positivity should be classified as multibacillary leprosy for treatment.

According to the Ridley scale, multibacillary patients have a BI of 2 or greater at any site, while paucibacillary patients have a BI of less than 2 at all sites (WHO, 1985).

Palermo *et al.* (1996) have reported that a significant reduction in epidermal cell proliferation in skin lesions in lepromatous leprosy is greater than in indeterminate leprosy lesions.

The nerve involvement showed that an indeterminate patient goes towards paucibacillary or multibacillary leprosy. Serial (1974) proposed that indeterminate form was the initial stage of leprosy which may either progress towards any of the polar form. Takahashi *et.al* (1991) once observed that ldt leprosy can change towards paucibacillary or multibacillary leprosy. Ridley (1971) reported a paucibacillary change of early leprosy cases with single skin lesions. On these occasions, immunologically based techniques should be of value since they combine both specificity and, chiefly, are not dependent on the presence of viable organisms (Mshana *et.al.*, 1982&1983).

Conclusion

It was concluded that correct diagnosis of indeterminate leprosy from other leprosy groups of spectrum could be made if results of clinical, histopathological, bacteriological and immunological were interpreted together. Detection of antibody levels appeared to be useful in the diagnosis and to confirm the indeterminate leprosy from other groups of spectrum. The early diagnosis and treatment of leprosy at indeterminate stage should be beneficial to reduce and to eradicate the leprosy from the community.

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How to cite this article:
Archana Singh Chauhan and Tabrez Ahmad. (2019). Study of Immunohistochemistry for mycobacterial antigen in skin biopsy for cases (IDT) and disease controls. Int. J. Adv. Res. Biol. Sci. 6(3): 184-190.
DOI: http://dx.doi.org/10.22192/ijarbs.2019.06.03.009