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Histopathological changes in leprosy and its correlation with IHC markers

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Abstract
Leprosy is a chronic granulomatous disease. It involves the psychological, socioeconomic dimensions of an individual. Histopathology is considered as an accurate method for diagnosing leprosy. Immunity is largely T-cell mediated, whereas deficiency of T-cells causes lepromatous leprosy. The relative proportion of T and B lymphocytes were studied using CD3 and CD20 immunohistochemistry markers (IHC). A Retrospective, prospective and observational study was carried from August 2016 to July 2019. The cases were divided based on histopathological findings. IHC markers CD3 was used to study T cell whereas CD20 was used to study B-cells. As per Ridley Jopling classification maximum cases belonged to indeterminate forms. Bacillary load of 4+ and above was seen in lepromatous leprosy. CD3 showed strong positivity in all types whereas CD20 showed more focal positive staining for lepromatous leprosy than tuberculoid and indeterminate forms.

Keywords: *Mycobacterium leprae*, CD3, CD20, granulomatous

Introduction
Mycobacterium leprae is responsible for chronic granulomatous disease. It is an endemic disease and the primary sites affected are the skin and nerves. The course of disease as well as the immune status of the individual is responsible for clinical presentation [1]. Leprosy is an example of bacteriological disease with an immunological background. In the 1966, a histological classification for leprosy was proposed by Ridley and Jopling. This classification included, indeterminate (IL) leprosy, polar tuberculoid (TT), borderline tuberculoid leprosy (BT), mid borderline leprosy (BB), borderline lepromatous leprosy (BL) and lepromatous leprosy (LL). [2] For field workers in 1988, the WHO devised a simplified classification, patients with skin smear negative for AFB are termed paucibacillary (PB), and those with skin smear positive are termed multibacillary (MB). [3] Immunity in leprosy is largely T-cell mediated, whereas deficiency of T-cell results in lepromatous leprosy. The orchestrated role of T-cells, macrophages, antigen presenting cells, such as dendritic cells is well known to be of primary importance in leprosy pathology. [4] Leprosy is considered by many as not merely a medical condition, but a condition encompassing psychological and socioeconomic dimensions. Leprosy is responsible for disfiguration in individuals. IHC markers CD3, CD20 are available at Bharati Vidyapeeth Medical College, Pune. IHC markers CD3 was used to study T cell whereas CD20 was used to study B-cells. This study was also done to evaluate the relative proportion of T and B lymphocytes in the lesions of leprosy.

Material and Methods
A retrospective, prospective and observational study was carried out on all successive cases sent to the Department of Pathology, for histological examination from August 2016 to July 2019. Cases received from August 2016 to July 2017 were studied retrospectively. Cases from August 2017 to July 2019 were studied prospectively. For retrospective evaluation, H&E and AFB test slides, requisition forms and clinical details were retrieved from the Pathology department. For prospective study biopsies taken from skin lesions sent in 10% buffered formalin were used. Findings of histopathological examination were compared to diagnose the different types of leprosy. IHC markers CD3 was used to study T cell whereas CD20 was used to study B-cells. IHC could be performed on 56 cases. Blocks of 5 cases were not available for IHC as they were used to prepare control slides for AFB-L stain.

Slit skin results were obtained from microbiology department. The data was analysed using SPSS software. The collected data was analysed using SPSS software. P value <0.005 was considered statistically significant.

Results

61 cases were evaluated in this study. 37 were males and 24 were from females. Male: female ratio was 1.54:1. Maximum patients belonged to the age group of 31-40 yrs.

Table 1: Distribution of cases according to Ridley-Jopling classification

Types of leprosy	Total number of cases
Indeterminate (IL)	23
Tuberculoid (TT)	7
Borderline tuberculoid (BT)	15
Borderline borderline (BB)	0
Borderline lepromatous (BL)	3
Lepromatous (LL)	13
Total	61

Table 2: Variants of lepromatous leprosy

Variants of lepromatous leprosy	Total number of cases
Histoid (HL)	2
Erythema Nodosum Leprosum (ENL)	3
Total	5

Changes in epidermis

All cases showed thinning of epidermis except 2 cases of IL which did not show any change. Focal loss and blunting of rete ridges was seen in 27 cases. Complete loss of rete ridges was seen in 19 cases. Normal appearance of epidermis was seen in 15 cases. Focal loss and blunting of rete ridges was commoner than complete loss of rete ridges. All cases showed normal thickness and appearance of keratin and normal granular layer. In LL, grenz zone was distinct while in TT, it was absent. In BL the grenz zone was focal and indistinct.

Changes in dermis

Thickened collagen bundles were seen in 9 out of 61 cases. All cases of TT showed granulomas reaching upto the basal layer of epidermis whereas in all cases of BT granulomas did not reach upto the basal layer of epidermis. The p value was 0.001, hence statistically significant. Periadnexal, perineural and perivascular granulomas and lymphocytic infiltrate were found in all cases of TT & BT leprosy.

Table 3: Granulomas in TT and BT

Types of leprosy	Granulomas reaching upto basal layer of epidermis	Granulomas not reaching upto basal layer of epidermis	Total	p value
TT	7	0	7	0.001
BT	0	15	15	
Total	7	15	22	

Table 4: Prominence of Arrector pilorum in different types of leprosy

Types of leprosy	Thickened/Prominent	Not thickened/ normal	Not included in the biopsy	Total
IL	14	6	3	23
TT	2	3	2	7
BT	8	4	3	15
BL	1	1	1	3
LL	2	7	4	13
Total	27	21	13	61

13 cases did not show arrector pilorum muscle in the biopsy material

Table 5: Slit skin examination was done in only 22 cases out of 61 cases

Bacillary index								
Type	No	Paucibacillary	Multibacillary					
			1+	2+	3+	4+	5+	6+
IL	7	7	-	-	-	-	-	-
TT	3	3	-	-	-	-	-	-
BT	3	1	2	-	-	-	-	-
BB	0	0	-	-	-	-	-	-
BL	1	-	-	-	-	-	1	-
LL	5	-	-	-	-	-	4	1
HL	2	-	-	-	-	2	-	-
ENL	1	-	-	-	-	-	1	-

Table 6: Correlation of AFB-L with bacillary index

AFB in slit skin preparations	No. of Cases	Type of Leprosy	AFB-L in tissue sections		Total No. of cases	p Value
Bacteriological Index (22/61)			AFB-L Seen	AFB-L Not Seen		
AFB Not detected	3	TT	0	3	3	0.003
AFB Not detected	1	BT	1	0	1	
AFB Not detected	7	IL	0	7	7	
1+	2	BT	0	2	2	
3+	1	BL	1	0	1	
4+	2	HL	2	0	2	
5+	5	LL	4	0	4	
		ENL	1	0	1	
6+	1	LL	1	0	1	
Total	22		10	12	22	

AFB-L on HP section stained by Fite Faracco was done on all 61 cases. AFB-L was detected in 13 cases of LL, 3 cases of BL and 7 cases of BT

Findings of IHC markers: CD3 & CD20 in leprosy cases

CD3: 55 cases showed strong positivity for CD 3 while 1 case of IL showed focal positivity

CD20: 21 cases showed focal positivity while 35 cases did not show CD20 positive lymphocytes. Strong positivity was not seen in any of the cases.

Table 7: CD20 in various types of leprosy

Diagnosis	Focally Positive	Negative	Total	p value
IL	6	17	23	0.001
TT	2	5	7	
BT	3	11	14	
BB	0	0	0	
BL	1	0	1	
LL	9	2	11	
LL (polar type)	5	2	7	
HL	2	0	2	
ENL	2	0	2	
Total	21	35	56	

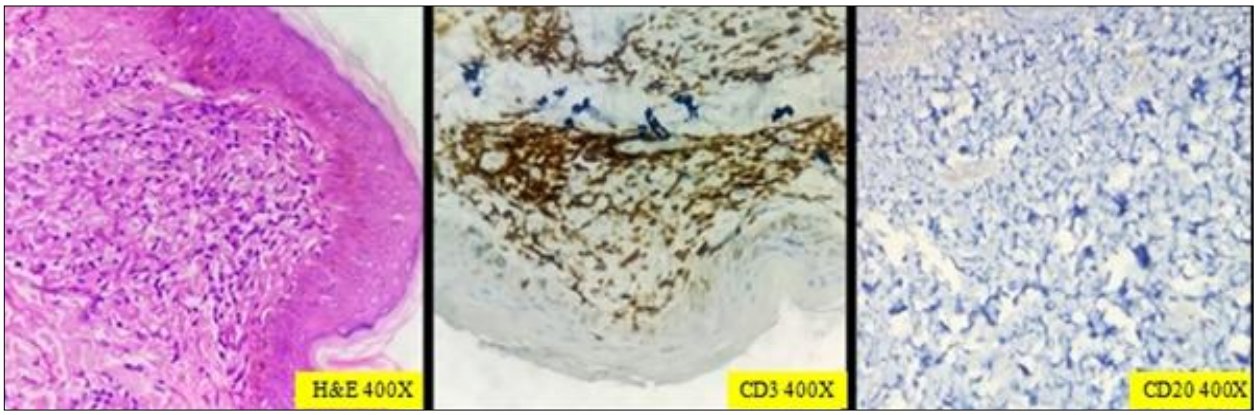

Fig 1: TT Granuloma extend end erode into the basal layer of epidermis. CD_3-strong positivity, CD20-negative

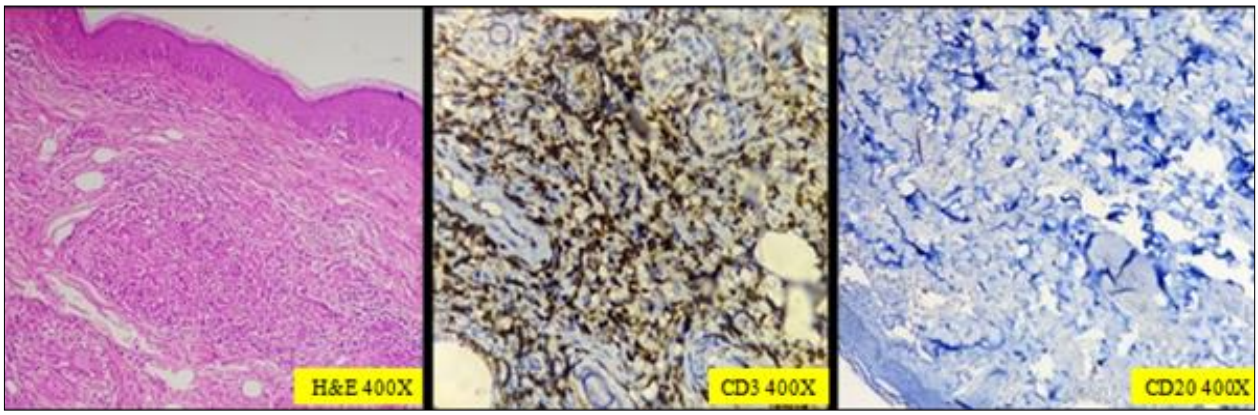

Fig 2: BT-Granuloma extend end erode into the basal layer of epidemis, CD_3-strong positivity, CD20-nagative

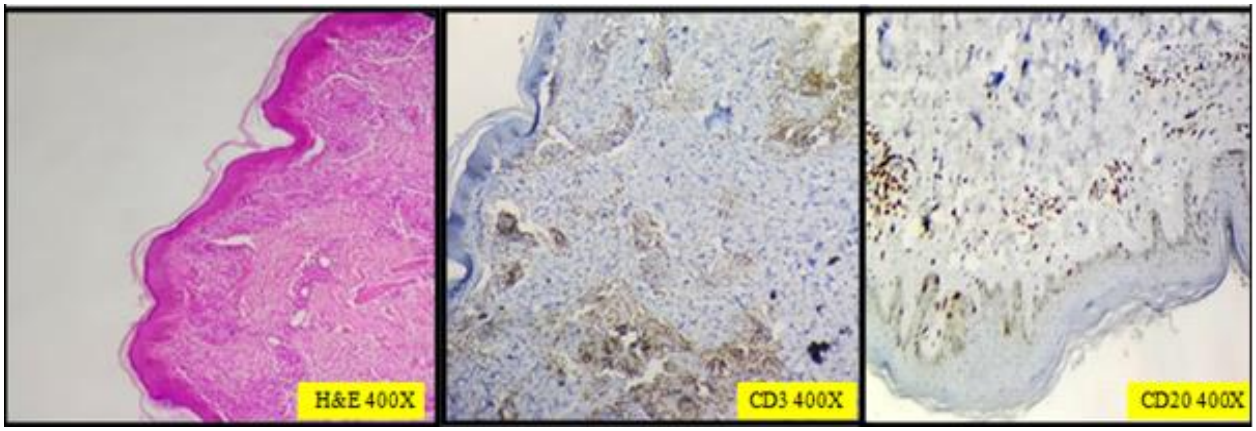

Fig 3: BL- Histiocytes may resemble epithelioid cells and shows foamy changes in cytoplasm. CD3-strong positivity, CD2O- focal positivity

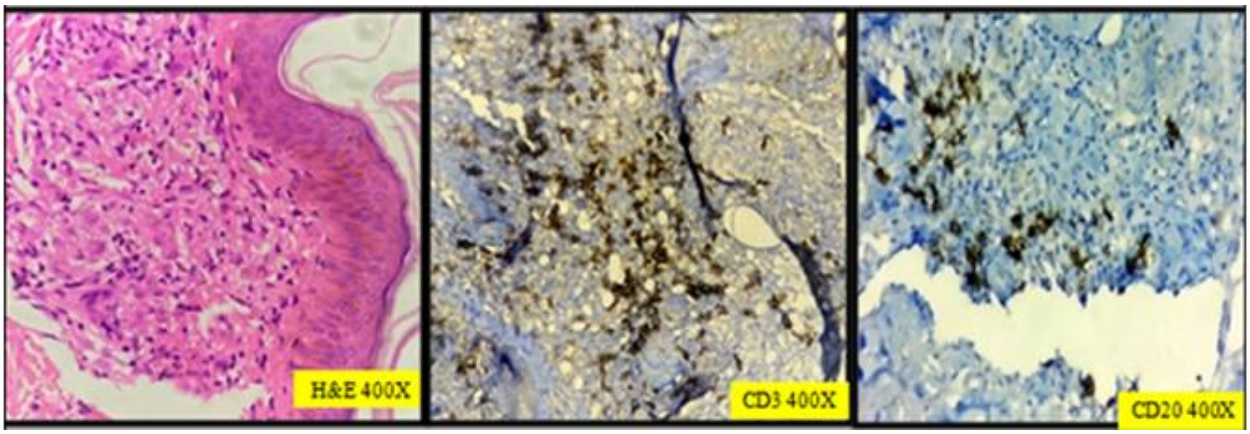

Fig 4: LL- Histiocytes may resemble epithelioid cells and shows foamy changes in cytoplasm. CD3-strong positivity, CD2O- focal positivity

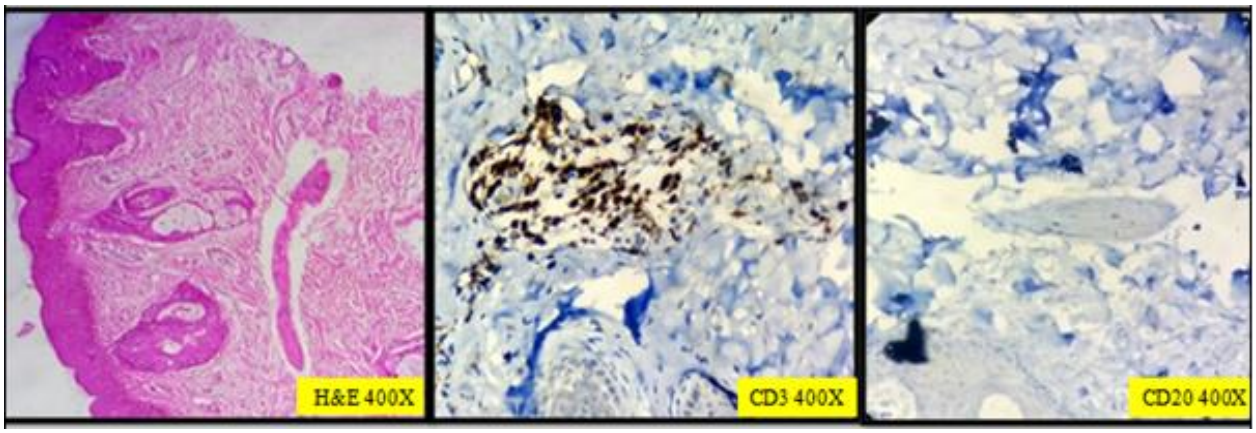

Fig 5: IL- Thickened arrector pilorum muscle seen. CD3-strong positivity, CD20- Negative

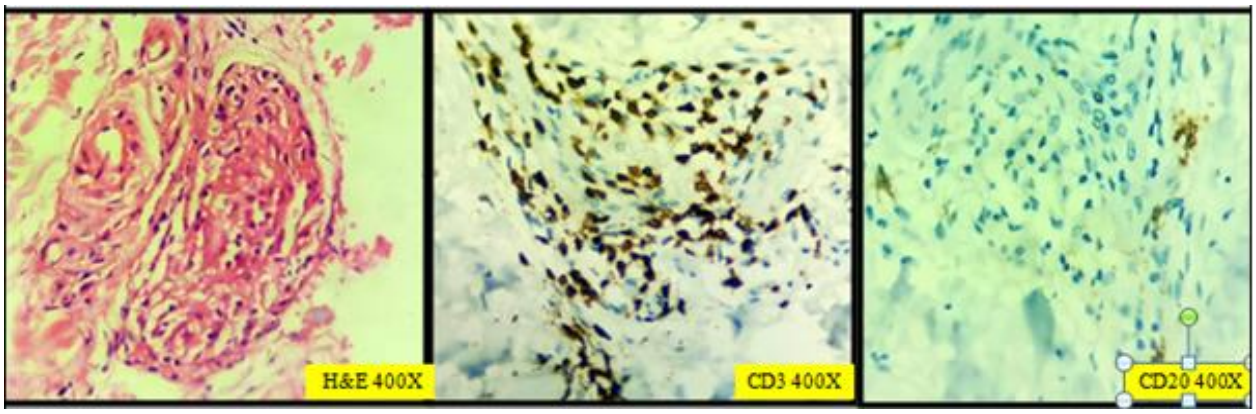

Fig 6: IL- Thickened arrector pilorum muscle seen. CD3-strong positivity, CD20- Negative

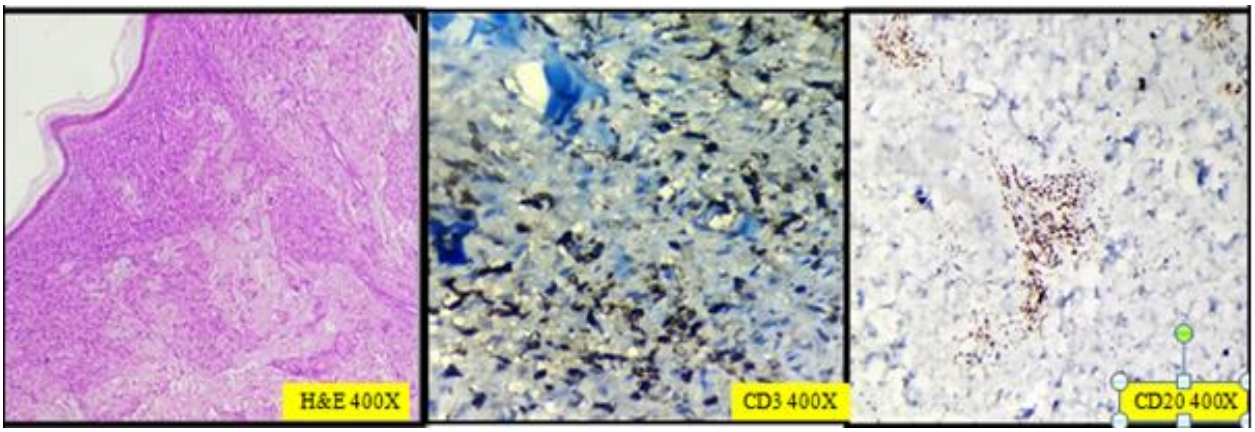

Fig 7: HL- Intertwining polyhedral to spindle shaped histiocytes seen in dermis. CD3-strong positivity, CD20-Focal positive

Discussion

A total of 61 cases of leprosy were included in this study, of which 23 were cases of indeterminate leprosy (IL), 7 cases of tuberculoid leprosy (TT), 15 cases of borderline tuberculoid leprosy (BT), 3 cases of borderline lepromatous leprosy(BL) and 13 cases of lepromatous leprosy(LL). In the present study, most common age group affected was the 4th decade. This was in contrary to the study done by Mathur *et al.* [5]. In the study done by Mathur *et al.* [5], majority of the cases belonged to the 3rd decade. In the study done by Mathur *et al.* [5] a total of 156 patients were studied, out of which 84 males and 72 were females. In the study done by Anuja Sharma *et al.* [6], the predominant population affected was males. This was in concordance with our study. In the present study, 22 belonged to tuberculoid polar leprosy and 16 belonged to lepromatous polar leprosy. Histopathology revealed epithelioid cells, Langhans giant cells, and lymphocytes toward the TT end of the spectrum and while foamy macrophages were abundant toward LL end of spectrum. In the case of LL, grenz zone was distinct while in TT, it was absent. In BL grenz zone was focal and distinct in all 3 cases. This was in concordance to the study done by Anusha *et al.* [7]. Thickened collagen bundles were seen in 9 out of 61 cases. Leprosy being a chronic granulomatous disease, increased collagenisation may be due the tissues response during healing. Some collagen bundles (I, II, III, IV) form linear fibrils stabilized by interchain hydrogen bonding. These fibrillar collagens form a major proportion of structures such as skin and take part in wound healing, regeneration and repair. In this study, out of 61 cases, 48 cases showed presence of arrector pilorum muscle in the biopsy. 27 out of 48 cases showed thickened/prominent arrector pilorum muscle. Although the total numbers of cases studied were small, prominent bundles of arrector pilorum muscle were seen in more than 2/3rd cases of IL. This feature may prove helpful in suspecting IL cases. None of the previous studies mentioned thickening of Arrector pilorum muscle. Hence, this change has not been compared with other studies. 7 cases of TT showed granulomas reaching upto the basal layer of epidermis whereas in cases of BT, all 15 cases showed dermal granulomas. This was in concordance with the study done by Anusha *et al.* [7]. Out of 61 cases in this study, bacteriological index was done on 22 cases. A low BI was seen in TT, BT and IL. BI was highest in LL types, similar to the observation made by Jopling[8] which shows that the bacilli are scanty or absent in BT, and are numerous in BL and LL [8]. If Globi are present in BL they tend to be small and unlike the large globi seen in LL. In this study, Fite faraco stain was positive for AFB-L in 23 skin biopsies and AFB were detected in 3 with BL, 13 with LL and 7 cases of BT. TT, IL cases did not show bacilli in Fite stain. This was in concordance with the study done by Manandhar U *et al.* [9], which showed similar findings. In this study the positive scoring for CD 20 was more in BL/LL patients as compared to BT/TT patients. The presence of more B cell towards the lepromatous pole in this study is consistent with higher antibody levels demonstrated in this form of leprosy. A strong staining for CD 3 was seen in all types of leprosy except for 1 case of IL showing focal positivity. This study was in concordance with the study done by Anand M *et al.* [10] which showed more B cells towards lepromatous pole and intense to moderate staining for T cells in BT, BL and LL cases. The findings in our study were contradictory to the study done by Fachin LR *et al* [11] which showed abundant CD20 lymphocytes in TT and high CD3 in TT than LL.

Conclusion

Histopathological examination proved to be an important method to help in accurate diagnosis and classification of leprosy. It still remains the gold standard. This study tried to find out the relative proportion of T and B lymphocytes in the lesions of leprosy using immunohistochemistry and showed that the positive scoring for CD 20 was more in BL/LL patients as compared to BT/TT patients. The presence of more B cell towards the lepromatous pole in this study is consistent with higher antibody levels demonstrated in this form of leprosy. A strong staining for CD 3 was seen in all types of leprosy except for 1 case of IL showing focal positivity.

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