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Micropathological changes in the sub-epidermal zone of normal appearing skin in leprosy

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Abstract

Leprosy is a chronic inflammatory disease caused by *Mycobacterium leprae*, which affects not only the peripheral nerves and skin but also various internal viscera through the hematogenous spread, especially in lepromatous cases. The micropathological changes in epidermis, nerves and skin appendages from lesioned skin reported by various authors but reports of involvement of apparently normal sites are few. We investigated skin biopsy material taken from 130 patients with clinically diagnosed leprosy. Biopsies were taken at least 10 cm away from site of lesion. Hematoxylin and Eosin staining and Harada's modified Allochrome method for acid-fast bacilli were applied for histological investigations. The pattern of leprosy among the patients were indeterminate in 53 cases (40.8%), tuberculoid in 29 cases (22.3%), borderline tuberculoid in 14 cases (10.8%), borderline leprosy in ten cases (7.7%), borderline lepromatous in nine cases (6.9%) and lepromatous leprosy in 15 cases (11.5%). The changes were seen in sub-epidermal zone of normal appearing skin in all type of leprosy, but involvement was greater at the lepromatous end of the spectrum compared to tuberculoid end. Acid-fast bacillus (AFB) was seen in subepidermal zone of normal appearing site. Presence of AFB is significant as far as dissemination and transmission of disease is concerned.

Keywords: leprosy, sub-epidermal zone, acid-fast bacillus, lepromatous leprosy, indeterminate leprosy.

Introduction

Leprosy, also known as Hansen's disease, is a chronic infectious disease that primarily affects the skin and peripheral nerves [1–3]. It is caused by *Mycobacterium leprae*. Leprosy expresses itself in different clinico-pathological forms depending on the immune status of the host [4]. A reliable diagnosis hinges around a good histopathological diagnosis and demonstration of bacilli in histopathological sections [5, 6]. We observed micropathological changes in the sub-epidermal zone of apparently normal skin of all types of leprosy. The involvement was more in cases at the lepromatous end of the spectrum compared to tuberculoid end.

Materials and Methods

Hundred and thirty patients with characteristic skin lesions of leprosy attending skin outpatient department, constituted the material for the study. Criteria's for patient's selection for study were:

Inclusion criteria

▪ All newly clinically diagnosed leprosy cases with characteristic skin lesions, with or without systemic symptoms.

Exclusion criteria

▪ Patient previously (defaulters and relapse) treated for leprosy.

▪ Association with other serious diseases such as HIV/AIDS, tuberculosis, lymphoma, leukemia, etc.

Incisional biopsies of all selected cases (after obtaining the written consent of patients) were taken at least 10 cm away from site of lesion. The biopsy was carried out under complete aseptic conditions. The site was thoroughly cleaned with sprit and approximately 0.2 mL of 1% lignocaine solution was injected with a hypodermic needle after the sensitivity was tested. Using a sterile B.P. knife, an elliptical piece of skin (size 1×0.5 cm) was removed. The biopsy was fixed in 10% formalin and processed for paraffin sectioning. The following staining methods were applied for histological investigations: Hematoxylin and Eosin; Harada's modified Allochrome method for acid-fast bacilli [7].

The work was approved by Ethics Committee of Subharti Medical College.

Data is represented in bar chart and *Z*-test for proportion (double sample) was used to evaluate the significant difference between different histological features at 1% level of significance for different leprosy forms.

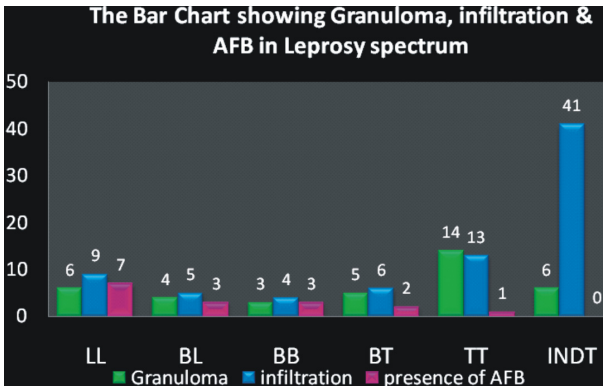
Results

A total of 130 patients were included in this study. Cases were taken from Meerut (UP) and nearby area present in northern India. Out of 130 cases, 98 were males (12 were less than 10-year-old) and 32 were females (three were less than 10-year-old).

Histopathological findings were graded into tuberculoid (TT), borderline tuberculoid (BT), borderline leprosy (BB), borderline lepromatous (BL) and lepromatous leprosy (LL) according to Ridley and Jopling scale [8]. Sections showing scattered non-specific lympho-histiocytic infiltration were classified as indeterminate leprosy [9]. The pattern of leprosy among the patients were indeterminate (INDT) in 53 cases (40.8%), tuberculoid (TT) in 29 cases (22.3%), borderline tuberculoid (BT) in 14 cases (10.8%), borderline leprosy (BB) in 10 cases (7.7%), borderline lepromatous (BL) in nine cases (6.9%) and lepromatous leprosy (LL) in 15 cases (11.5%) (Table 1).

Table 1 – ***Histological features of sub-epidermal zone in normal appearing skin of leprosy cases***

Leprosy forms	HE staining		Harada's modified Allochrome method
	Granuloma	*Infiltration*	*Presence of AFB*
Lepromatous leprosy (LL) n=15, 11.5%	6 (40%)	9 (60%)	7 (46.7%)
Borderline lepromatous (BL) n=9, 6.9%	4 (44.4%)	5 (55.5 %)	3 (33.3%)
Borderline leprosy (BB) n=10, 7.7%	3 (30%)	4 (40%)	3 (30%)
Borderline tuberculoid (BT) n=14, 10.8%	5 (35.7%)	6 (42.8%)	2 (14.3%)
Tuberculoid (TT) n=29, 22.3%	14 (48.3%)	13 (44.8%)	1(3.4%)
Indeterminate (INDT) n=53, 40.8%	6 (11.3%)	41 (77.3%)	–



The micropathological changes were seen in sub-epidermal zone of normal appearing skin in all type of leprosy. Micropathological changes include granuloma, infiltration and presence of acid-fast bacilli (bar chart). The commonest form of leprosy observed was indeterminate type. However, out of 130 cases, 96 were towards tuberculoid end of spectrum (INDT, TT and BT) and 34 were towards lepromatous end of spectrum (BB, BL and LL).

HE stained sections were used to see infiltration and granuloma while sections stained with Harada's modified Allochrome method were used for demonstration of acid-fast bacilli.

Infiltration of varying degree (Figure 1) was seen in almost all cases, from lepromatous to tuberculoid end of spectrum. Formation of granuloma (Figure 2) and presence of acid-fast bacilli (Figure 3) were also seen.

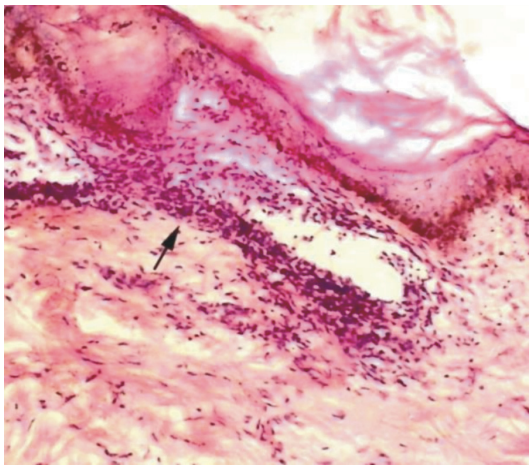


Figure 1 – ***Photograph showing minimal sub-epidermal infiltration in an indeterminate leprosy from apparently normal skin (HE staining, 100×).***

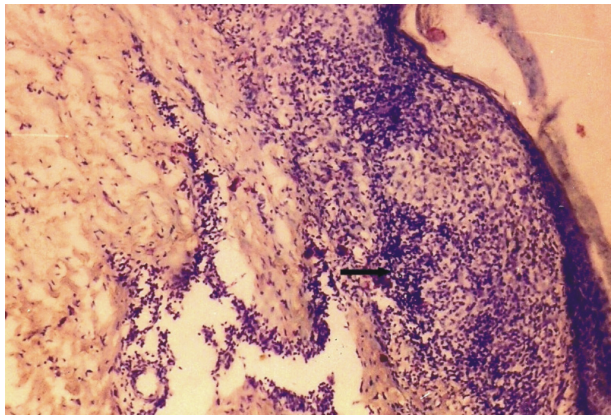


Figure 2 – ***Photograph of sub-epidermal granuloma in tuberculoid case from normal looking skin (HE staining, 100×).***

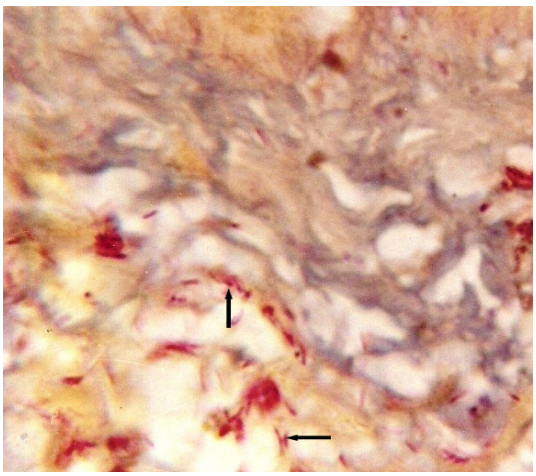


Figure 3 – ***Photomicrograph showing the group of bacilli in the sub-epidermal zone in normal looking skin of a lepromatous case. Bacilli group is identified in the figure by arrows (Harada's modified Allochrome method, 400×).***

The *Z*-test for proportion (double sample) was used to test the significant difference between different histological features i.e. granuloma and infiltration (Table 2), granuloma and AFB (Table 3) and infiltration and AFB (Table 4) at 1% level of significance for different leprosy forms. A significant difference was observed between histological features of different forms of leprosy i.e. $p<0.01$.

Table 2 – ***Significant difference between granuloma and infiltration between various leprosy forms***

Leprosy forms	*Z*-value	Zcrit. α=0.01	*p*-value
Lepromatous leprosy (LL)	3.61	2.58	<0.01
Borderline lepromatous (BL)	4.10		
Borderline leprosy (BB)	3.28		
Borderline tuberculoid (BT)	4.12		
Tuberculoid (TT)	3.33		
Indeterminate (INDT)	7.59		

P<0.01 shows significant difference between granuloma and infiltration for various leprosy forms.

Table 3 – ***Significant difference between granuloma and presence of AFB between various leprosy forms***

Leprosy forms	*Z*-value	Zcrit. α=0.01	*p*-value
Lepromatous leprosy (LL)	3.61	2.58	<0.01
Borderline lepromatous (BL)	5.21		
Borderline leprosy (BB)	2.91		
Borderline tuberculoid (BT)	3.00		
Tuberculoid (TT)	6.01		
Indeterminate (INDT)	7.88		

P<0.01 shows significant difference between granuloma and presence of AFB for various leprosy forms.

Table 4 – ***Significant difference between infiltration and presence of AFB between various leprosy forms***

Leprosy forms	*Z*-value	Zcrit. α=0.01	*p*-value
Lepromatous leprosy (LL)	4.18	2.58	<0.01
Borderline lepromatous (BL)	3.59		
Borderline leprosy (BB)	2.91		
Borderline tuberculoid (BT)	5.00		
Tuberculoid (TT)	8.03		
Indeterminate (INDT)	12.00		

P<0.01 shows significant difference between infiltration and presence of AFB for various leprosy forms.

Discussion

Leprosy is a problem associated with social stigma in India. It is more prevalent in Bihar, West Bengal, Orissa and Uttar Pradesh as compared to other states of India [10]. We had taken 130 cases from Meerut and nearby areas (Uttar Pradesh) for our study. There are reports on equal or higher prevalence of females from Thailand, Nigeria, Uganda and Gambia [11–13]. Sex difference varies with age as children show either less or no sex variation in prevalence compared to adults. It might be because existence of same biological and physiological factors in childhood [14]. However, in this study male preponderance was seen even in the first decade age. There were only three female children whereas male children were 12.

Reports on histological studies of uninvolved site in leprosy are few. In these studies, either normal skin studied was very close to the lesion or the number of cases was less. Gougerot H and Degos R [15] observed the histological changes in the uninvolved skin just 2 to 4 cm away from the lesion. Ganapati R *et al.* [16] noted minimal or negligible sub epidermal infiltration in the apparently normal skin of tuberculoid and borderline tuberculoid cases. In their study, the normal skin was from the periphery of the lesion, just 0.5 to 2 cm away from the lesion. Rea TH *et al.* [17] observed the histological changes in the uninvolved skin 7 cm away from the lesion in 31 lepromatous and three borderline lepromatous patients. Mascaro JM *et al.* [18] found the infiltration in the uninvolved skin in the opposite side of the lesion on the face in one patient. Tutakne AM *et al.* [19] investigated histologically the normal skin 0.5 cm away from borderline tuberculoid and tuberculoid lesions. Verma NR *et al.* [20] observed the histological changes in the normal skin at the periphery of the lesion in 40 cases. The present study differs from the other studies as biopsy of uninvolved skin was taken at least 10 cm away from lesion and 130 patients investigated.

Chacko CJ *et al.* [21] observed the nasal mucosal involvement in tuberculoid cases and thought that the dissemination in the tuberculoid lesions occurs at the earliest stage. Verma NR *et al.* [20] refuted the presence of any micro pathological lesion in the normal skin from the periphery of the tuberculoid lesions in eight cases. Singh K *et al.* [22] found the unequivocal presence of "indeterminate lesion" in the apparently normal skin from the symmetrically opposite side of the tuberculoid lesion in three cases. They proposed that tuberculoid form is also generalized systemic disease. Dharmendra (1979) [23] was of opinion that tuberculoid form might be initial vascular spread till the activation of cell mediated immunity (CMI), but once it is mounted the vascular spread stops. The bacilli, which might have been deposited during this initial vascular spread, either disintegrate or remain as such. Dharmendra (1983) [24] again stressed that micropathological lesion in tuberculoid cases were due to initial vascular spread. These lesions will be arrested soon after CMI is activated. He opposed labeling tuberculoid form as generalized or systemic disease. The number of tuberculoid cases included in our study was 29 which showed the sub-epidermal infiltration in 13 (44.8%), also favoring Dharmendra opinion that initial vascular spread responsible for pathological lesions.

The apparently normal skin of cases towards lepromatous end of spectrum (LL, BL and BB) showed well-developed sub-epidermal granuloma. The granuloma in mid-borderline lesion was composed of epitheloid cells with few lymphocytes and macrophages. In borderline lepromatous it was composed of mainly lymphocytes with macrophages, whereas lepromatous cases showed the presence of foamy cell granuloma with numerous bacilli. These characteristic features were similar to that described by Ridley DS and Jopling WH [8]. Bedi TR *et al.* [25] observed the foamy cell granuloma in the immune zones like scalp, axilla and groin in 5% of LL cases. Katoch VM *et al.* [26] reported the presence of small granuloma and less

number of bacilli compared to that of the lesion in the immune zone groin in four out of 22 LL cases. Singh K *et al.* [22] observed no granuloma in the normal skin from the opposite side of LL lesion. In present study, the sub-epidermal zone was getting narrower with enlarging granuloma. The granulomatous lesion was seen in all forms of leprosy but more towards lepromatous end of spectrum as compared to tuberculoid end but there is significant difference present between granuloma and infiltration in all forms of leprosy.

It is reasonable to expect the presence of bacilli in the normal skin of LL, BL and BB forms of leprosy, where bacteremia plays a major role in dissemination. Ganapati R *et al.* [16] found the presence of bacilli in the normal skin very close to the lesion. Rea TH *et al.* [17] demonstrated bacilli in normal appearing skin of 96% LL cases, but failed to demonstrate bacilli in BL cases. Kaur S and Kumar B [27] observed the bacilli in 75% in the normal looking skin of BL cases by slit and scrape skin smear method. Singh K *et al.* [22] found the presence of AFB in the normal skin in 66.6% of BL cases. Periaswamy V [28] could demonstrate the bacilli in the periphery of borderline leprosy, Ramu G and Ramanujam K [29] could find the bacilli in five out of 28 cases by smear method in normal looking skin 0.5 to 2.0 cm away from the borderline lesion. In the present study, we also observed bacilli more in lepromatous end of spectrum.

Demonstration of bacilli in normal appearing skin in BT, TT and INDT form was difficult. It was even more difficult to find bacilli in sub-epidermal clear zone. Klingmüller G [30] suggested that the factors like lack of optimal growth temperature, ultraviolet rays by direct sunlight, compression by granuloma and presence of phagocytic fibroblasts might be responsible for lack of bacilli in sub-epidermal clear zone. Most of the scientists did not find bacilli in sub-epidermal zone in paucibacillary form including Ganapati R *et al.* [16] who reported the absence of bacilli in the normal skin of the TT, BT and maculoanaesthetic leprosy. Tutakne AM *et al.* [19] and Verma NR *et al.* [20] did not find bacilli in the normal skin close to periphery of the lesion in tuberculoid cases, but in the present study, bacilli were observed in two cases of BT and one case of TT. No bacilli observed in INDT form.

Sub-epidermal zone involvement exists in the normal appearing skin of all types of leprosy. Moreover, the degree of lesion increases with decrease in the immunity from tuberculoid to the lepromatous end of spectrum. AFB was seen in sub-epidermal zone of normal appearing site, their presence is significant as far as dissemination, and transmission of disease is concerned. It may be due to phagocytic activity of keratinocytes, which engulf bacilli; hence, possibility of discharge of leprosy bacilli from intact skin, even without ulceration should be seriously considered [31].

Conclusions

From this study, it is concluded that sub epidermal zone involvement exists in the normal appearing skin of all type of leprosy. Moreover, the degree of lesion increases with decrease in the immunity from TT to LL end of spectrum. Acid-fast bacilli were seen in significant number in sub epidermal zone as far as transmission of disease is concerned.

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