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Short Communication

Histopathological Diagnosis of Leprosy Type 1 Reaction with Emphasis on Interobserver Variation

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Upgrading type1 lepra reaction or reversal reaction (RR) is an acute inflammatory complication of leprosy and a disparity exists between clinicians and pathologists for diagnosing a RR. Inter-observer variations among pathologists also compound this problem as no universally agreed diagnostic criteria exist. 120 biopsies and H&E stained slides were assessed by 3 pathologists. The pathologists were blinded to the clinical diagnosis and to each other's observations. Each pathologist assigned a likelihood of reaction by their histopathological observations as definitely reaction, probable reaction and no reaction. Clinicopathological correlation and interobserver agreement was analyzed statistically. Discordance between clinical and histopathological diagnosis was seen in 30.8% by pathologist 1(P1), 23.7% by pathologist 2 (P2) and 34.5% by the pathologist 3 (P3). Dermal edema, intragranuloma edema and epidermal erosion were consistent findings by all observers. Definite reaction was seen in 54.2% of cases by P1, 53.3% by P2 and 34.5% by P3. Kappa statistics for strength of agreement showed good agreement between 3 pathologists with P1 (κ=0.83), P2 (κ= 0.61), P3 (κ=0.62). RR are underdiagnosed on histopathological examination but this study shows that dermal edema, edema within the granuloma and partial obliteration of grenz zone by granuloma are reliable clues to diagnose a RR on histopathology.

Key words: Leprosy, Reversal Reaction, Type 1 Reaction

Introduction

Leprosy remains a health problem in different parts of the world. At the end of first quarter of 2013, 189018 patients were affected by leprosy (prevalence rate 0.32 per 10000 population) as compared to 2012 where it was 181941 (0.34 per 10000) (WHO 2013). Leprosy type 1 reaction also called as upgrading reversal reaction (RR) is a delayed type hypersensitivity reaction in borderline forms of leprosy spectrum and is characterized by increased cell mediated immunity with influx of CD4+ T cells

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and macrophage activation. RR present as immunemediated episodes of acute inflammatory sequel that can cause serious complications like nerve damage and deformity. RR complicate approximately one-third of patients with borderline leprosy during the course of disease (Britton and Lockwood 2004). In view of wide variations in clinical presentation of leprosy RR, histopathological examination is an important and useful tool for supporting the clinical diagnosis. Due to lack of universally accepted criteria for histological diagnosis of RR and paucity of available literature on histopathology of RR, we designed this study to histopathological observations in 120 cases of clinically diagnosed leprosy and assess interobserver variability among three pathologists in diagnosing a RR.

Materials and Methods

This is a prospective study where 120 patients clinically diagnosed as upgrading type 1 leprosy reaction at the Department of Dermatology, Venereology and Leprology of Safdarjung Hospital in Delhi over a three-year period from 2010-2013 were included. Patient selection was done as per guidelines and after taking approval from the institutional ethical committee of Safdarjung Hospital. The clinical inclusion criteria for upgrading type 1 leprosy reaction were the occurrence of erythema and edema in previous leprosy lesions, appearance of new lesions, lesional and/or nerve tenderness with or without nerve abscess formation. The patient presenting with reaction for the first time, while on treatment or had completed MDT were included. The exclusion criteria was that reactions involving purely nerves without any skin lesions, those clinically resembling type 2 reaction and patients on immunosuppressant drugs were excluded from the study. Sixty non-reaction biopsies from borderline leprosy patients who did not have any documented past or present evidence of reaction served as controls. The patient age, sex and Ridley-Jopling leprosy classification, presence or absence of edema and peripheral nerve examination for nerve thickening were noted along with details of MDT provided (details not shown).

Skin biopsy and Histopathologic examination

Four millimeter punch biopsy or were taken by applying local anesthesia and maintaining strict aseptic conditions from the typical reactional skin lesion. All three pathologists involved in this study were given a proforma in which they had to record their findings which included (a) dermal edema, (b) edema within the granuloma, (c) epidermal erosion by granuloma or lack of subepidermal grenz zone, (d) lymphocytes within the granuloma and (e) changes in giant cell shape and size besides any other findings they wanted to report. Before recording their observations it was mutually agreed between the 3 pathologists that dermal edema will be defined as splaying of the dermal collagen fibers with pallor and increased vasculature. Edema within the granuloma was defined as when granulomas are not compact and show loosening and separation of epithelioid cells, histiocytes and lymphocytes by extracellular fluid. Epidermal erosion was defined as destruction of the basal layer of epidermis by the granulomatous infiltrate in addition to obscuring of the subepidermal zone of dermal collagen. Large to bizarre shaped giant cells and lymphocytes within the granuloma were some other parameters. Granuloma fraction (GF), was calculated by each of the pathologists.

The H&E stained slides were coded and same batch of slides were given for their observations to each of the 3 pathologists who were blinded to the clinical information and the histological findings of the each other for their observations. The pathologists were finally asked to give a diagnosis as no reaction, probable reaction

and definite reaction for each case studied. The criteria followed were that to call it a definite reaction more than 3 histological parameters should be present in the biopsy. To call it a probable reaction at least two parameters should be present and when none of the five parameters were present it was called as no reaction.

Statistical Analysis

All the histological findings of the three pathologists were entered in an excel sheet and analyzed by SPSS software v17. Spearman's rank correlation coefficient was calculated as a measure of association between individual histological parameters and clinical reaction status. For each pathologist histological parameters predictive of RR were analyzed using logistic regression analysis. Kappa statistics was applied for measuring the strength of agreement between the three observers. Kappa statistic value of <0.20 was considered poor agreement, 0.21-0.40 was considered fair, 0.41-0.6 was moderate and 0.61-8.0 was considered good and 0.8-1.0 was considered very good agreement. All comparisons were considered statistically significant at P value <0.05.

Results

Out of 120 cases 88 were males and 32 were females. The Ridley-Jopling classification for the cases was Borderline tuberculoid (BT) in 90, Midborderline (BB) in 20 and Borderline lepromatous (BL) in 10. Out of 120 patients, 36 patients were on MDT at the time of presentation.

The observations of the histological parameters, GF calculation and the diagnosis of a definite RR by each of the three pathologists are shown in Table 1. Definite RR was observed the maximum by the first pathologist while no reaction was seen the most by the third pathologist. Clinicopathological discordance was 30.8%, 23.7% and 34.5% by the three observers respectively.

Table 1 : Histopatholgical parameters reported by each pathologist

S.No	Histopathological Parameter	Pathologist 1 N=120 (%)	Pathologist 2 N=118 (%)	Pathologist 3 N=113 (%)
1	Epidermal erosion	42 (35)	38 (32.2)	32 (28.3)
2	Dermal edema	7 2 (60)	67 (56.7)	65 (57.5)
3	Intragranuloma edema	75 (62.5)	88 (74.5)	76 (67.2)
4	Intragranuloma lymphocytes	56 (46.6)	84 (71.1)	85 (75.2)
5	Giant cell size	19 (15.8)	29 (24.5)	27 (23.8)
6	Granuloma Fraction			
	0.0-0.33	21	23	20
	0.34-0.66	38	37	40
	0.67-1.0	61	58	53
7	T1R on histology			
	Definite T1 R	65 (54.2)	63 (53.3)	39(34.5)
	Probable T1R	18 (15.0)	27 (23.0)	35 (31.0)
	No T1R	37 (30.8)	28 (23.7)	39 (34.5)

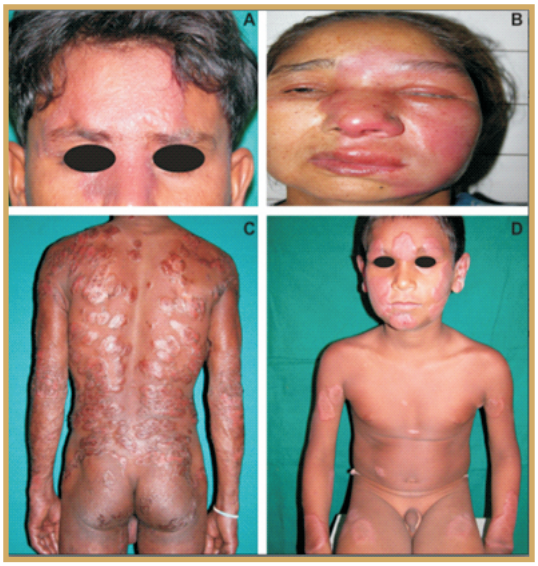

Figure 1 : (A) BT leprosy patient with (A) erythematous plaque on face and forehead, (B) facial edema, (C) BB leprosy in RR showing multiple indurated plaques with ulceration, (D) BL leprosy in RR showing thick discrete plaques.

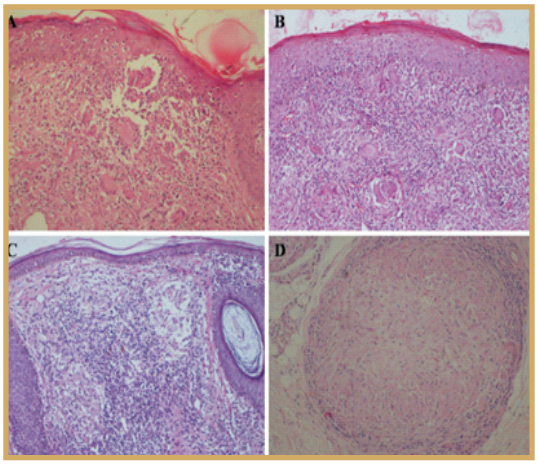

Figure 2 : Photomicrograph showing (A) dermal granuloma invading epidermis, (B) lack of subepidermal grenz zone with many large giant cells, (C) dermal edema and (D) loose epithelioid granuloma with intragranuloma edema and lymphocytes. (H&E, x 200)

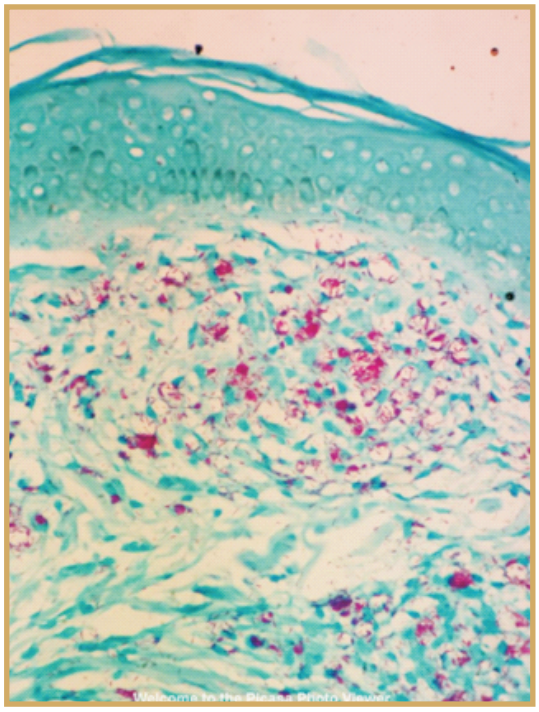

Figure 3. Photomicrograph showing Borderline lepromatous lepsrosy with scant perivascular infiltrate of histiocytes and lymphocytes and abundant acid fast bacilli within the granuloma. (Fite-Faraco stain, x 400)

Table 2 summarizes the Spearman's coefficient of variation and its statistical significance between the three observers. The most consistent histological parameters those were seen by all pathologists were epidermal erosion by the granuloma (Fig 2A), loss of subepidermal grenz zone (Fig 2B), dermal edema (Fig 2C) and edema & lymphocytes within the granuloma (Fig 2D). P2 and P3 also observed dermal edema and intragranuloma edema as most consistent finding when diagnosing a reaction. For assessing the inter-observer agreement, Kappa statistics was applied and an overall good agreement was seen between the three pathologists P1

Table 2 : Histological diagnoses of RR given by pathologists for clinical diagnosis of RR

S.No	Parameter	Pathologist 1		Pathologist 2		Pathologist 3	
		Sp Coeff	P-value	Sp Coeff	P-value	Sp Coeff	P-value
1	Epidermal erosion	0.601	<0.001	0.569	<0.001	0.198	0.03
2	Dermal edema	0.810	<0.001	0.743	<0.001	0.528	<0.001
3	Intragranuloma edema	0.643	<0.001	0.722	<0.001	0.392	<0.001
4	Intragranuloma lymphocytes	0.492	<0.001	0.618	<0.001	0.322	<0.001
5	Giant cell size and shape	0.292	0.001	0.432	<0.001	0.263	0.005

Table 3 : Strength of agreement between pathologists

No	Parameter	P1 vs P2 (κ)	P1 vs P3 (κ)	P2 vs P3 (κ)
1	Dermal edema	0.74	0.68	0.70
2	Intragranuloma edema	0.72	0.57	0.67
3	Intragranuloma lymphocyt	0.53	0.34	0.60
4	Epidermal invasion	0.72	0.57	0.62
5	Large groups of Giant cells	0.62	0.61	073
	T1R diagnosis	0.83	0.61	0.62

P1: pathologist 1, P2: pathologist 2, P3: pathologist 3

(κ=0.83), P2 (κ=0.61), P3 (κ=0.62) in diagnosing a RR as shown in Table 3.

Discussion

A wide variation in clinical presentation of leprosy in reaction makes histopathological examination an important tool in supporting the clinical diagnosis of a reaction. There are no universally agreed histological criteria for diagnosing a RR. Confirming a clinical diagnosis of RR is important as these patients once confirmed to be in RR need treatment for reducing acute inflammation with corticosteroids and for preventing the nerve damage. Ridley and Radia (1981) first described the histological features of RR and divided them into four phases proposing that edema is the most prominent feature while giant cell formation occurs as a late event (Ridley 1969). Sehgal et al (1986 and 1990) emphasized in their studies about the presence of dermal edema and loose and disorganized granuloma as important features in an upgrading reaction. Fine et al (1993) showed in their report that there could be interobserver variations in histopathological diagnosis of clinically suspected leprosy due to subjective interpretation and similar variations could also exist in diagnosing a RR. Lockwood et al (2008) studied 99 patients with clinically diagnosed RR and 52 controls and 4 pathologists independently reviewed these slides. They concluded that reactions were underdiagnosed on histology with 32-62% of clinically diagnosed RR being given a histological diagnosis consistent with a reaction. They observed that the key morphological features for diagnosing a RR are

dermal edema, intra-granuloma edema, giant cell size and number and HLA-DR expression in epidermis. Thomas et al (2013) documented that dermal edema, intragranuloma edema and giant cell size are the most sensitive parameters for diagnosis of leprosy type 1 reaction.

Our study showed in contrast to study published previously Lockwood et al (2008) that there would be reactions diagnosed histologically that had not been apparent clinically. Though there were some differences among the three pathologists, P1 and P2 were consistent in diagnosing a reaction while P3 diagnosed the reactions the least. Kappa statistics (Table 3) showed good agreement between pathologist 1 and pathologist 2 (κ=0.83) and between P1 and P3 (κ=0.61) and P2 and P3 was also good (κ=0.62).

Focal or partial obscuring of grenz zone was seen in many cases of type 1 reaction unlike in tuberculoid leprosy which shows complete lack of subepidermal grenz zone. Another observation in our study was the frequent presence of lymphocytes within the granuloma in reactional biopsies unlike in non-reaction leprosy which show lymphocytes surrounding the epithelioid granuloma. This finding had also described by (Adhey et al 2012). All the three observers in our study were consistent with their observations of dermal edema and intra-granuloma edema. Large size and variations in shape of multi-nucleated giant cells was also subjective and varied among the three pathologists. Pathologist 1 additionally reported clustering of these large-sized Langhans giant cells in upper dermis and they were not uniformly distributed in the granulomatous infiltrate. Cree et al (1995) measured GF in leprosy reactions by planimetry and showed that GF fell in 15/22 patients on treatment of leprosy with MDT but increased in 5/22 cases which showed increase in GF. These five cases again showed a fall in GF when treated with steroids. This could possibly be explained by an increase in immunity in an upgrading reaction leading to influx of lymphocytes and macrophage activation resulting in formation of giant cells and the granuloma.

To conclude, type 1 reaction occur frequently during the course of leprosy and they sometimes may show clinicopathologic discordance. In our observation, the sensitive histological parameters which support the diagnosis of RR are dermal edema, edema within the granuloma, obscuring of grenz zone with epidermal erosion and additional clues like lymphocytes within the granuloma and large, clustered and sometimes bizarre shaped multinucleated giant cells. Inter-observer variations occur between pathologists possibly because of the subtle nature of these histological findings and these variations could be due to quantitative rather than qualitative nature.

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References

1. Adhey V, Dongre A and Khopkar U (2012). A Retrospective Analysis of Histopathology of 64 Cases of Lepra Reactions. *Indian J Dermatol.* **57**: 114-117.
2. Britton WJ and Lockwood DN (2004). Leprosy. *Lancet*. **363**: 1209-1219.
3. Cree IA, Coghill G, Subedi AMC et al (1995). Effects of Treatment on the Histopathology of Leprosy. *J Clin Pathol*. **48**: 304-307.
4. Fine PEM, Job CK, Lucas SB et al (1993). Extent, Origin and Implications of Observer Variation in the Histopathological Diagnosis of Suspected

Leprosy. *Int J Lepr Other Mycobact Dis*. **61**: 270-282.

5. Lockwood DNJ, Lucas SB, Desikan KV et al (2008). The Histological Diagnosis of Leprosy Type 1 Reactions: Identification of Key Variables and an Analysis of the Process of Histological Diagnosis. *J Clin Pathol.* **61**: 595-600.
6. Ridley DS (1969). Reactions in leprosy. *Lep Rev.* **40**: 77-81.
7. Ridley DS and Radia KB (1981). The histological course of reactions in borderline leprosy and their outcome. *Int J Lepr Other Mycobact Dis*. **49**: 383-392.
8. Sehgal VN, Bhattacharya SN and Jain S (1990). Relapse or Late Reversal Reaction? *Int J Lepr Other Mycobact Dis.* **58**: 118-121.
9. Sehgal VN, Gautam RK, Koranne RV et al (1986). The Histopathology of Type I (Lepra) and Type II (ENL) Reactions in Leprosy. *Ind J lepr.* **58**: 240-243.
10. Thomas M, Ponnaiya J, Emmanuel M et al (2013). Type 1 Reaction in Leprosy-A Histopathological Analysis. *Indian J Lepr.* **85**: 1-4.
11. World Health Organization (2013). Global leprosy situation. *Wkly Epidemiol Record.* **35**: 365-380.

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