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RT: Histo-morphological variants of leprosy A cross sectional study on correlation between histo-morphological types of leprosy with bacilloscopic index

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Abstract
Introduction: This study has aimed hypothesizing the presence of an unbalance between the TLR1 and TLR2 expressions associated to high bacillary loading and IL-10 expression in leprosy reactions, which, consequently, are favorable to survival of bacillus and the occurrence of these events.
Materials and Methods: All the case diagnosed as leprosy were evaluated by Fite Faraco special stain and reported for bacilloscopy index according to reference guideline as below.
Result: Out of 62 cases suspicious for clinically diagnosed leprosy, maximum number of cases were observed in the age group of 31 to 40 years (40%). Among various anatomical site for cutaneous presentation of leprosy in maximum number of cases, the lesions were observed in upper extremity.
Conclusion: Bacteriological examination and bacilloscopy index add onto the morphological diagnosis and helps to categorise multibacillary and pauci bacillary leprosy. We recommend it to avoid false over and under diagnosis of leprosy cases.

Keywords: histomorphological, correlation, bacilloscopy, leprosy

Introduction

The type 1 reaction (T1R), subdivided in upgrading and downgrading, is a delayed hypersensitivity reaction against components of *M. leprae*, whose the affected clinical forms are borderline tuberculoid (BT), borderline borderline (BB), and borderline lepromatous (BL) [1]. The upgrading and downgrading reactions are clinically indistinguishable, characterized by the presence of oedema and erythema in preexisting skin lesions, appearance of new skin lesions with classic inflammatory signs, and neuritis associated with sensory and motor alterations [2]. On the other hand, such reactions may be differentiated by histopa- thology, the profile of the immunological response, and tem- porality of the occurrence of these events [2]. The upgrading reaction, also called reverse reaction, occurs after administration of multidrug therapy (MDT), in which the type 1 helper (Th1) cytokine pattern (interleu- kin-1β [IL-1β], tumor necrosis factor-alpha [TNF-α] IL-2, and interferon-gamma [IFN-γ]) is found in patient lesions, in addition to elevation of TNF-α, IFN-γ, and IL-17F in the serum of these patients and other markers such as interferon gamma-induced protein 10 (IP-10), vascular endothelial growth factor (VEGF), and chemokine 10 (CXCL10) [3-6] The T1R guarantees resistance against *M. leprae*, leading to migration in the clinical spectrum of the disease of those bor- derline individuals to the tuberculoid pole, reducing, finally, the bacilloscopic and morphological indices [7]. On the contrary, the downgrading reaction occurs before MDT and after treatment in relapse cases, representing an immunological activity directed against nonessential anti- genic determinants of *M. leprae* survival. Thus, it may be observed in downgrading reaction the increase in the number of bacilli, B lymphocyte levels, and immunoglobulin gamma (IgG) antibodies, besides the low levels of natural killers and T cells [2, 8, 9] Furthermore, the immunological profile of this reaction allows evasion mechanisms of the bacillus favoring the migration of borderline individuals towards the lepromatous leprosy (LL) pole in the clinical spectrum of the disease [7, 10]. The diagnosis of leprosy is based on different clinical parameters which involve detailed examination of skin lesions and peripheral nerves along with slit-skin smear examination, histopathological examination, and demonstration of acid-fast bacilli [11]. The present study was carried out to assess the concordance between clinical and

histopathological diagnosis in cases of leprosy using Ridley–Jopling scale. The disease has a 2- to 5-year course for paucibacillary patients and a 5- to 10- year course for multibacillary patients.13 Humans are the main natural reservoir of the bacillus. MB patients are considered the main source for infection in transmission cycle. Although there is evidence of the presence of *M. leprae* in skin lesions, breast milk, environment and animals, the main route of transmission for *M. leprae* is the respiratory tract [12, 14, 15]. During disease evolution, reactions might occur that, without proper treatment, can lead to severe damage in the peripheral nerve trunks, originating physical disabilities and sequelae, the main reason for the stigmatization caused by the disease [16]. The Ziehl-Neelsen and Kinyoun methods remain reliable ways to visualize the presence of acid-fast bacteria in human exudates smears. However, a more recent adaptation of the Kinyoun staining method, the Fite-Faraco method, is currently the preferred staining procedure to identify *M. leprae* in human tissues. The main adaptation in the Fite-Faraco method is the dilution of the solvent xylene in the vegetable oils used during the deparaffinization step because *M. leprae* is much less acid- and alcohol-fast than M. tuberculosis and thus can easily be missed in the examination of the slide. Therefore, by means of gene expressions, serological data, and a causal model, this study has aimed hypothesizing the presence of an unbalance between the TLR1 and TLR2 expressions associated to high bacillary loading and IL-10 expression in leprosy reactions, which, consequently, are favorable to survival of bacillus.

Materials and Methods

The present cross-sectional study was conducted in the Department of Dermatology at a tertiary health-care teaching institute in India. Skin biopsies of all suspected cases of Hansen's disease received over a period of 3 years (April 2015–May 2018) were included in the study. Hematoxylin and eosin and Fite-Faraco stained sections of all cases were reviewed. All the case diagnosed as leprosy were evaluated by Fite Faraco special stain and reported for bacilloscopy index according to reference guideline as below. When searching for the leprosy bacillus in smears or tissue samples, Ridley and Jopling established that a negative result should only be reported following the examination of at least 100 microscopic oil immersion fields, as recommended for tuberculosis [14]. For that reason, the correct histological analysis is time - consuming and laborious. Antibody titers were expressed as direct values of optical density and subsequently subjected to statistical normalization for a percentage scale that maintained the ratio between differences in antigen expression levels. The number of bacilli identified by this method, together with the clinical and histopathological features, helps classify the disease form. The Ridley and Jopling classification of leprosy utilizes the bacilloscopic index, varying from a score of 0 to 6, and is based on a logarithmic scale in which 0 represents the absence of bacillus; 1+ represents 1–10 bacilli in 100 fields; 2+, the presence of 1–10 bacilli in 10 fields; and 3, 4,5, and 6+ represent the identification of 1–10, 10–100, 100–1000, and >1000 bacilli per field respectively. In addition, wherever available the corresponding slit-skin smear was also reviewed.

Result

Out of 62 cases suspicious for clinically diagnosed leprosy, maximum number of cases were observed in the age group of 31 to 40 years (40%). Among various anatomical site for cutaneous presentation of leprosy in maximum number of cases, the lesions were observed in upper extremity. This cases were biopsied to confirm histomorphology. [Table 1].

Table 1: Correlation between age group and anatomical lesions in clinically suspicious cases of leprosy

Age group (years)	Upper extremity	Head and neck	Trunk and back	Lower extremity	Total * (out of 50)
0-20	--	--	--	--	0
21-30	02	01	01	02	06(12%)
31-40	09	03	02	06	20 (40%)
41-50	10	02	02	04	18 (36)
>50	01	02	01	02	06 (12%)
Total	22(44%)	8(16%)	6(12%)	14(28%)	--

Out of 62 biopsy examined 50 cases were confirmed histo-morphologically as cases of various types of leprosy. Higher number of type of leprosy observed were borderline tuberculoid leprosy (30%) followed by tuberculoid leprosy (28%). All the cases diagnosed as various types of leprosy were undergone for FiteFaraco special stain to confirm the diagnosis and to get bacillary load. In one case diagnosed as histoid leprosy, bacilloscopic index was 7+. It is observed mean bacilloscopy index is higher in cases of lepromatous leprosy whereas lower in tuberculoid leprosy. 2 cases were histomorphologically diagnosed as tubercular leprosy and on FF stain observed bacilloscopic index 0+. [Table 2].

Table 2: Correlation between histomorphologicaltype of leprosy with bacilloscopic index (Fitefaraco stain)

S. No.	Histomorphological type of leprosy	Number of cases	Mean bacilloscopic index
1	Lepromatous leprosy	8(16%)	5.81
2	Borderline lepromatous leprosy	6(12%)	4.2
3	Intermediate leprosy	4(8%)	2.70
4	Borderline tuberculoid leprosy	15(30%)	1.5
5	Tuberculoid leprosy	14(28%)	1
6	Indeterminant leprosy	--	--
7	Histoid leprosy	2(4%)	8
8	Histomorphological findings other than leprosy	13(26%)	--

Discussion

It primarily affects the skin and the peripheral nerves [19]. It can be progressive and can cause permanent damage to the skin, nerves, limbs, and eyes.18 In the present study, the cases were classified according to Ridley– Jopling classification into indeterminate leprosy (I), TT, BT, mid-borderline (BB), BL, and LL. Cases of histoid leprosy, lepra reactions, and ENL were also included in the study. The male preponderance for leprosy noted in our study was also been shown in other studies like Manandhar *et al.* [17] and Vargas-Ocampo [20]. This might be attributed to increased chances of exposure due to increased job-related mobility [17]. In the present study, clinico-histological correlation was observed in 62% of cases. The concordance percentage for clinico-histological correlation was almost similar to the studies conducted by Moorthy *et al*., [21] Kalla *et al*., [22] Bhatia *et al*., [2] and Kar *et al*. [24] The cellular characteristics in leprosy lesions are related to the immunological modulation of the patient. Hence, different grades of

modulation affect the host defensive response and result in different types of clinicopathological pictures [25]. Selection of the site for biopsy plays an important role in histopathological diagnosis since clinically dissimilar lesions biopsied from the same patient can show different types of histopathology [26]. As expected Fite-Faraco stain was positive in 100% cases of LL type or MB type of leprosy. Slit-skin smear test helps in establishing an early diagnosis of Hansen's disease. However, this test has high specificity but low sensitivity and as many as 70% of leprosy cases are smear negative [27]. The results of slit-skin smear correlated with that of Fite-Faraco-stained sections in LL spectrum of leprosy. For lesions toward TT pole of leprosy, we found higher positivity rate on Fite-Faraco-stained histological sections as compared to SSS. This was probably because of the fact that increased step sections of the paraffin-embedded block increased the chances of detection of bacilli in PB cases. Bacillary index in granuloma was also found to be higher than that of slit-skin smear by Ridley who opined that slit-skin smear reflected density at a particular foci while sections also took into account the size of the lesion along with density.28 In the present study, 43 cases of clinically diagnosed leprosy were discordant. In most of these cases (36/43), the findings on histopatho- logical examination were nonspecific. However, confirmed diagnosis was established in 7/43 cases comprising of granulomatous lesion (2/43), polymorphous light eruption (2/43), retiform hemangioendothelioma (1/43), pityriasis rosea (1/43), and epidermal atrophy (1/43). The challenges in eradication are delay in detecting new patients persisting discrimination against people affected by leprosy, and limited impact on transmission of leprosy. India continues to account for 60 percent of new cases reported globally each year. The NLEP in its recent evaluation have acknowledged that there are cases occurring in the community and detection capacity is not matching the level and intensity of disease occurrence. Basic investigations such as skin smear services need to be reintroduced in the leprosy programme of India, as this bacteriological test is often found as useful as advanced PCR techniques. In a study conducted in a leprosy research centre to assess drug resistance, findings have shown the value of reintroducing skin smear examination for confirmation/classification of leprosy as it was found reliable in detecting bacilli in 43% of the patients, including 24% of paucibacillary leprosy patients.29 From its introduction in 1982 to till date, the same three drugs constitute MDT for leprosy, and with emerging resistance to these drugs, there is a need to expand the repertoire of drugs to treat leprosy. Only when all proven cases of Hansen's disease undergo regular follow-up after treatment and are diligently screened for bacillary load before labeling them as disease free, we shall be able to realize our dream of making our country free from the scourge of leprosy.

Conclusion

Clinical detection and morphological diagnosis of early lesions remain challenging, and the histological findings should always be interpreted in correlation with clinical findings. Thus, we conclude and hypothesized, in reactional groups, a possible signaling pathway favoring the formation of TLR2/2 homodimers, association of TLR2/6, and consequently, greater expression of IL-10, which may favor bacillary survival and the occurrence of these events. The understanding of this unbalanced response may lead us to novel therapeutic strategies to prevent leprosy reactions. In our study carried out at tertiary care hospital, borderline tuberculoid and tuberculoid cases were reported with higher incidence. Bacteriological examination and bacilloscopy index add onto the morphological diagnosis and helps to categorise multibacillary and pauci bacillary leprosy. We recommend it to avoid false over and under diagnosis of leprosy cases.

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