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ORIGINAL ARTICLE

A Cross Sectional Study on Correlation between Histomorphological Type 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 FiteFaraco 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 leprosycases.

Keywords: Histomorphological, correlation, bacilloscopy**,** leprosy

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INTRODUCTION

The delayed hypersensitivity reaction known as type 1 reaction (T1R), which is classified into upgrading and downgrading, is caused by components of Mycobacterium leprae. The clinical forms of T1R include borderline tuberculoid (BT), borderline borderline (BB), and borderline lepromatous (BL).1 The presence of oedema and erythema in preexisting skin lesions, the emergence of new skin lesions with characteristic inflammatory symptoms, and neuritis linked to sensory and motor changes are the clinically identical features of both upgrading and downgrading reactions.2. On the other hand, histology, the immunological response's profile, and the timing of these events can all distinguish various kinds of reactions.2.After multidrug therapy (MDT), the upgrading reaction, also known as the reverse reaction, takes place. This involves the presence of the type 1 helper (Th1) cytokine pattern (interleukin-1β [IL-1β], tumour necrosis factor-alpha [TNF-α], IL-2, and interferon-gamma [IFN-γ]) in patient lesions, along with elevated levels of TNF-α, IFN-γ, and IL-17F in the patients' serum as well as other markers like interferon gamma-induced protein 10 (IP-10), vascular endothelial growth factor (VEGF), and chemokine 10 (CXCL10).3 = 6 Finally, the bacilloscopic and morphological indices decrease because the T1R ensures resistance against M. leprae, which causes migration in the clinical spectrum of the disease in those borderline individuals to the tuberculoid pole.7. Conversely, in relapse instances, the downgrading reaction happens after treatment and before to MDT; it is an immune response against non-essential antigenic determinants of M. leprae survival. As a result, in addition to the low numbers of T cells and natural killers, the downgrade reaction may show an increase in bacilli, B lymphocyte counts, and immunoglobulin gamma (IgG) antibodies.2. 8, 9 Additionally, this reaction's immunological profile permits the bacillus to evade mechanisms, which favours borderline people' migration towards the lepromatous leprosy (LL) pole in the clinical spectrum of the disease.7-9 A variety of clinical characteristics, including a thorough inspection of skin lesions and peripheral nerves, a slit-skin smear test, a histological examination, and the presence of acid-fast bacilli, are used to diagnose leprosy.Eleven The current study used the Ridley-Jopling scale to evaluate the degree of agreement between the clinical and histological diagnoses in leprosy cases. For paucibacillary patients, the disease's course lasts two to five years, while for multibacillary people, it lasts five to ten years.Thirteen The primary natural reservoir of the bacillus is humans. Patients with MB are thought to be the primary source of infection in the cycle of transmission. The respiratory tract is the primary mode of transmission for M. leprae, despite indications of its prevalence in animals, the environment, breast milk, and skin lesions.12–14, 15 As the condition progresses, reactions may happen that, if left untreated, can cause serious damage to the peripheral nerve trunks, resulting in physical disabilities and sequelae. These physical limitations are the primary cause of the disease's stigma.sixteen When examining human exudate smears for the presence of acid-fast bacteria, the Ziehl-Neelsen and

Kinyoun procedures continue to be dependable visual aids. The Fite-Faraco approach, a more modern variation on the Kinyoun staining technique, is currently the recommended staining technique to identify M. leprae in human tissues. Since M. leprae is substantially less acid- and alcohol-fast than M. tuberculosis and can therefore be easily overlooked throughout the slide examination process, the key change in the Fite-Faraco method is the dilution of the solvent xylene in the vegetable oils employed during the deparaffinization step. Thus, this study has attempted to hypothesise the existence of an imbalance between the TLR1 and TLR2 expressions associated with high bacillary loading and IL-10 expression in leprosy reactions, which, consequently, are favourable to survival of bacillus and the occurrence of these events. It has done this by using gene expressions, serological data, and a causal model.

MATERIALS AND METHODS

The present cross-sectional study was conducted in the Departments of Dermatology at a tertiary health-care teaching institute in Jaipur 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 thestudy. Hematoxylin and eosin and Fite-Faraco stained sections of all cases were reviewed. All the case diagnosed as leprosy were evaluated by FiteFaraco 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 normaliza- tion 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 bacilliper field respectively. . In addition, wherever available the corresponding slit-skinsmearwasalsoreviewed.

RESULT

The age range of 31 to 40 years old accounted for the largest number of cases (40%), out of 62 cases that were suspect for clinically confirmed leprosy. The upper extremities were the most often observed anatomical region for cutaneous leprosy presentations, with lesions being found there in the majority of patients. The histomorphology of this case was confirmed through biopsies. See 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%)	--

Fifty instances were proven histomorphologically as cases of different forms of leprosy out of the 62 biopsies investigated. Tuberculoid leprosy (28%), followed by borderline tuberculoid leprosy (30%), was the most common kind of leprosy seen. FiteFaraco special stain was used on all individuals identified as having different forms of leprosy in order to confirm the diagnosis and determine the bacillary burden. One case with a bacilloscopic index of 7+ was identified as having histoid leprosy. The mean bacilloscopy index has been found to be lower in tuberculoid leprosy cases and higher in lepromatous leprosy cases. According to histomorphological analysis, two instances had tubercular leprosy, and bacilloscopic index 0+ was seen using FF stain. [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

Peripheral nerves and skin are the main areas affected.19 It may worsen over time and result in irreversible harm to the eyes, limbs, skin, and nerves.eighteen According to the Ridley–Jopling classification, the cases in the current investigation were divided into five categories: indeterminate leprosy (I), TT, BT, mid-borderline (BB), BL, and LL. The study also included cases of lepra reactions, ENL, and histoidleprosy.The male majority for leprosy shown in our study was also observed in previous investigations, including Vargas-Ocampo and Manandhar et al. 17.Twenty This could be explained by greater exposure opportunities brought on by more mobility in the workplace.17. Clinico-histological association was found in 62% of the cases in the current investigation. The clinico-histological correlation concordance % was nearly identical to that of the research done by Bhatia et al.,2 Kar et al., Kalla et al.,21 Moorthy et al., and 22 Kalla et al.24- The patient's immune modulation is connected to the cellular features of leprosy lesions. Therefore, varying degrees of modulation impact the host defensive response and give rise to distinct clinicopathological images.25, Since clinically diverse lesions biopsied from the same patient can display different forms of histology, the biopsy site selection is crucial to the histopathological diagnosis.26. Fite-Faraco stain was positive in 100% of instances of leprosy, either MB type or LL type, as predicted. The slit-skin smear test aids in the early detection of Hansen's illness. As many as 70% of leprosy cases are smear negative, and this test has a high specificity but a low sensitivity.27, The LL spectrum of leprosy showed a correlation between the results of the slit-skin smear and the Fite-Faraco-stained sections. Fite-Faraco-stained histological sections for lesions at the leprosy TT pole showed a higher positivity rate than SSS. This was most likely caused by the paraffin-embedded block's enlarged step sections, which raised the likelihood of finding bacilli in PB instances. Additionally, it was discovered that the bacillary index in granulomas was larger than that of slit-skin smears. Ridley believed that slit-skin smears indicated density at specific foci, but sections also considered the size of the lesion in addition to density.28, Forty-three cases of clinically confirmed leprosy were discordant in the current investigation. The results of the histopathological study were vague in the majority of these instances (36/43). However, in 7/43 cases—granulomatous lesion (2/43), polymorphous light eruption (2/43), retiformhemangioendothelioma (1/43), pityriasisrosea (1/43), and epidermal atrophy (1/43)—a confirmed diagnosis was made. Detecting new cases of leprosy is slow, discrimination against those affected by the disease persists, and eradication efforts have little effect on the disease's ability to spread. India continues to be the country responsible for 60% of newly reported cases worldwide annually. In a recent evaluation, the NLEP admitted that instances are happening in the community and that the level and intensity of disease occurrence do not correspond with detection capacity. The leprosy project in India needs to reinstate basic investigations like skin smear services, as these bacteriological tests are frequently found to be just as helpful as sophisticated PCR procedures. Reintroducing skin smear examination for confirmation/classification of leprosy is valuable, as evidenced by the findings of a study conducted in a leprosy research centre to assess drug resistance. The study found that 43% of the patients, including 24% of paucibacillary leprosy patients, had bacilli detected with reliability.29, The same three medications have been part of the leprosy MDT since its launch in 1982, however as resistance to these medications develops, more medications are needed to treat leprosy. We will never be able to achieve our goal of ridding our nation of the leprosy plague until all confirmed cases of Hansen's disease receive routine follow-up following treatment and are carefully checked for bacillary load before being declared disease free.

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 the seevents. The understanding of this unbalanced response may lead us to noveltherapeuticstrategiestopreventleprosyreactions. 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 categorisemultibacillary and pauci bacillary leprosy. We recommend it to avoid false over and under diagnosis of leprosycases.

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