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EXPRESSION OF S100 PROTEIN AND CD1A MARKER IN LESIONS OF LEPROSY

Pathology

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ABSTRACT

Background: Leprosy first described in ancient Indian texts is a nonfatal, chronic infectious disease caused by Mycobacterium leprae. Histological diagnosis is deemed the gold standard for diagnosis of leprosy. Immunohistochemical markers play an important role in monitoring of progression of the disease and effect of treatment. SlOO protein was produced robustly and continuously in macrophages from Lepromatous leprosy patients' lesions. Distinguishing characteristics of LCs suggested that their expression of langerin may enable them to capture certain antigens when they reach the epidermal layers, and that at least some of these antigens might be delivered to CD1a-restricted T cells. In the present study, both S100 protein and CD1a expression has been studied on 38 cases of leprosy cases for assessing the hypothesis. **Objectives:** Histopathological differentiation of types of leprosy and correlate the expression of S100 protein and CD1a antibody. **Methodology:** This study was conducted in Department of Pathology, Gandhi Medical College, & Associated Hospitals, Bhopal between 1st January 2020 to 30th June 2021. Skin biopsies received in Department of Pathology, Gandhi Medical College and Hamidia Hospital, Bhopal. Information was taken from requisition forms received in Department of Pathology. History of cases was also taken through case files and interview. The samples were processed for histopathology evaluation and immunohistochemistry staining for S100 and Cd1a. **Results:** In our study, out of 38, 52.63% cases were diagnosed to be Indeterminate leprosy followed by 23.68 cases of Tuberculoid leprosy , Borderline tuberculoid and Lepromatous leprosy cases were 13.16% and 10.53% in numbers respectively. Cross tabulation for S100 positivity as per histological diagnosis showing that all Lepromatous leprosy cases gave positive expression for this IHC marker whereas all Tuberculoid leprosy cases were negative. Out of 5 cases of BT, 3 were S100 positive and 2 out of 20 cases of IL gave positivity. All Tuberculoid cases (except 1) gave positivity for CD1a and all Lepromatous cases were negative. Out of 20 IL cases, 9 gave positivity. All 5 BT cases gave positivity. **Summary:** Owing to treatment, early or late presentations, immunological status of the host, histo-morphology may vary as well, however, it remains to be the gold standard of diagnosis. Our study evaluated the role IHC markers namely S100 and CD1a as an ancillary technique to support histopathological diagnosis and guide early and more specific diagnosis in early leprosy cases, assess host's immune status as well as hint towards transition through the spectrum.

KEYWORDS

Leprosy, Histopathological diagnosis, Immunohistochemistry

INTRODUCTION:

Leprosy is a nonfatal, chronic infectious disease caused by Mycobacterium leprae, whose clinical manifestations are largely confined to the The skin, the peripheral nervous system, the upper respiratory tract, the eyes, and the testes are all affected. [1] Leprosy is known, since ancient times as “Kushtaroga” The discovery of lepra bacillus by Gerhard Henrik Armaeur Hansen in 1873 opened a new vista in the understanding of the disease. The unique tropism of M. leprae for the peripheral nerves (from large nerve trunks to microscopic dermal nerves) and certain immunologically mediated reactional states are the major causes of morbidity in leprosy. Although it was the first bacterium to be etiologically associated with human disease, M. leprae remains one of the few bacterial species that still has not been cultivated on artificial medium or tissue culture.[2] Presentation may vary from an insignificant skin lesion to extensive disease causing profound disability/deformities. [2] Histopathological study of leprosy is very important in understanding the disease. Clinical diagnosis of early leprosy lesions offer difficulties even to experienced dermatologists and leprologists. Histological diagnosis is the gold standard for diagnosis of leprosy.[3] For further categorisation, immunohistochemical markers play an important role. SlOO protein was produced robustly and continuously in macrophages from polar, subpolar, and borderline Iepromatous leprosy patients' lesions. In contrast, the lesions of polar and subpolar tuberculoid leprosy had very few cells that were immunoreactive for SlOO protein ('SlOO-i-') in the granulomas in the dermis.[3]

The intracellular residence of Mycobacterium leprae makes cytolytic activity of the macrophages essential for the destruction of the organisms[4-11]. Genetic factors also play a role in the type of lymphocytic response to M. leprae organisms [12-15]. Paul Langerhans, as a medical student, identified Dendritic cells resident in the skin, specifically the epidermis, so-called Langerhans cells (LCs) have been shown to be the only cells in the epidermis to express MHC class II molecules under normal conditions [16,17]. LCs move to regional lymph nodes after antigen collection and are extremely effective at delivering MHC class II–restricted peptides to T cells [18,19]. These T cells then return to the skin to take part in inflammatory reactions there. These findings imply that LCs play a significant role in the skin's innate immune response, triggering adaptive T cell responses locally [20], a member of the family of group 1 CD1 proteins (CD1a, CD1b, and CD1c), which share the capacity to present microbial lipid antigens to T cells [21-24]. LCs are the only cells known to express langerin (CD207) [25], a C-type lectin that is sufficient to induce the formation of Birbeck granules, pentilaminar endosomal structures specific to LCs [26]. In the present study, both S100 protein and CD1a expression has been studied on 38 cases of leprosy cases for assessing the aforementioned hypothesis.

Methodology:

This study was conducted in Department of Pathology, Gandhi Medical College, & Associated Hospitals, Bhopal between 1st January 2020 to 30th June 2021. Samples were received as formalin fixed tissue samples/biopsies of skin received. Information or history was taken from requisition forms received in Department of Pathology. History of cases was also taken through case files and interview. The samples were processed for histopathology evaluation and immuno histochemistry staining for S100 and CD1a.

IHC ASSESSMENT

Staining score for S100: (Positive / Negative)

Staining score for CD1a expression: (Positive/Negative)

An index of positivity (IP) was calculated which will represent the percentage of labelled cells in 1000 counted cells in the 40x microscope field:

Percentage:

0: <10%
1: 10-25%
2: 26-50%
3: >50%

Intensity of staining will be scored as:

0: Negative
1: Weak
2: Moderate
3: Strong

Heterogeneity:
0: Negative
1: Marked
2: Moderate
3: Mild

RESULTS:
Out of 38 cases, 71.1% were males and 28.9% were females. Among males mean age was 42.11 years and 41.55 years in females. Out of the total cases, most (71.1%) had anaesthesia over the hypopigmented patches whereas 28.9% had sensation intact. 24 had palpable, inflamed peripheral nerves. Overall only 2 patients had visible hand and foot deformity. In most cases (36) there was no deformity clinically evident. WHO grades leprosy based on two parameters: Anaesthesia and Visible deformity as follows:

- Grade 0: No anaesthesia, no deformity
- Grade 1: Anaesthesia present, no deformity
- Grade 2: Both present

In our study, 25 patients fell under Grade 1 category, 11 in Grade 0 and only 2 were in grade 3.

20 cases were diagnosed to be Indeterminate leprosy (which in an early lesion of leprosy with non specific morphology) followed by 9 cases of Tuberculoid leprosy, Borderline tuberculoid and Lepromatous leprosy cases were 5 and 4 in numbers respectively.

Out of 38 cases, 29 were negative (i.e., <10% positivity) for S100 expression that comprised of all tuberculoid cases and few of BL and IL. A single case gave strong positivity(>50%) which was lepromatous leprosy Equal number of cases (4 each) gave mild (10-25%) and moderate(26-50%) positivity which comprised of a combination of IL and BL cases. 29(76.3%) cases were negative for S100 while remaining (23.7%) gave weak to strongly intense staining. 29 out of 38 cases showed negative immunoexpression for S100. Amongst those with positive staining 4 each showed marked and moderate heterogeneity whereas a single case showed mild heterogeneity.

Out of 38 cases, 16 were negative (i.e., <10% positivity) for CD1a expression that comprised mostly of all Lepromatous leprosy cases. 3 cases gave strong positivity(>50%) which were tubercloid leprosy cases mostly. 8 cases gave mild (10-25%) and 11 cases gave moderate(26-50%) positivity which comprised of a combination of IL, BL and TT cases. 16(42.1%) cases were negative for CD1a while remaining (57.9%) gave weak to strong intense staining. 16 out of 38 cases showed negative immunoexpression for CD1a. Table 1 shows P value for lepromatous leprosy to give positive immunostaining for S100 came out to be less than 0.05 and is therefore significant.

All Lepromatous cases gave positivity and all tuberculoid cases gave negativity. Table 2 shows all Tuberculoid cases (except 1) gave positivity and all Lepromatous cases gave negativity for CD1a. Out of 20 IL cases, 9 gave positivity. all 5 BT cases gave positivity. Association of CD1a expression with TT cases has a positive and significant correlation (p value less than 0.05). Pearson coefficient of 0.8 shows significant linear association.

Table 1: Correlation of S100 expression LL cases.

		S100		total
		Negative	Positive	
Histological diagnosis	LL	0	4	4
	TT	9	0	9
Total		9	4	13

Table 2: Correlation of CD1a expression in TT cases.

		S100		total
		Negative	Positive	
Histological diagnosis	LL	4	0	4
	TT	1	8	9
Total		5	8	13

DISCUSSION:
In the present study, 38 clinically suspected and diagnosed cases of leprosy selected over the study period in a tertiary care centre. The study was aimed at assessing the expression of S100 protein and CD1a in histopathologically diagnosed cases of leprosy in order to accentuate the assigning of a spectrum more specifically. Detailed history and examination findings were obtained as per the proforma.

Mean age of patients irrespective of gender was approximately similar in the present study well in concordence to other studies. Majumdar et al [96] stated majority patients to be in the age group of 20-40 years (productive age group) as similarly found in the present study.

Noorden SK11[32] and Majumdar et al [96] observed that the dominance in males is because of their lifestyle which is associated with greater risks of acquiring the infection and also the social taboos and customs which account for underdetection of leprosy among females as lesser numbers report to the hospital for treatment. In the present study as well out of 38 cases 27 were males and only 11 were females.

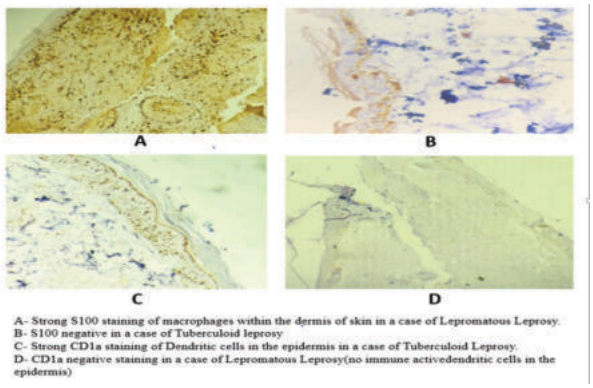
In the present study, most patients belonged to lower socio-economic class (as per Modified Kuppuswamy socio-economic scale) in positive concordense with the study conducted by Majumdar et al [96].

The study of skin biopsies continues to remain a standard tool in the classification and diagnosis of leprosy. The 38 skin biopsies in the present study were classified according to histopathological features and subsequently correlated with the clinical features in order to arrive at an accurate diagnosis.

In the present study, all the cases of LL gave moderate (2+) to strong (3+) positivity for S100 protein whereas were in toto negative for CD1a expression. On the contrary, TT cases did not express S100 whereas 8 out of 9 i.e., 88.9% cases gave positive immunoexpression for CD1a. In a similar study conducted by Cuevos Santos et.al.[3] all the cases falling under the spectrum of lepromatous leprosy showed intense positivity for S100 protein, however, none of the cases (of LL, BL, BT or TT) gave any immunoexpression for CD1a. It is suggested that macrophages are derived from blood monocytes[3] which then have S100 positive and S100 negative sub- populations. There is a proposed transition of the sub-population from one pole to the another.[3] Mohandas et.al.[101] conducted a study using S100 staining to assess the number of dendritic cells and nerve damage in leprosy. He observed membranous positivity for S100 in lepromatous leprosy cases similar to that observed by Azadeh et. al.[84].

Azadeh et.al.[84] in his study evaluated the immune status of patients from both the polar spectrum by assessing the functionality of langerhan cells and dermal dendritic cells (antigen presenting cells) using S100 expression. He concluded that functional LCs index was low towards LL spectrum compared to TT. Miranda et.al. conducted a study to study increased recruitment of LCs. The results showed a high number of LCs in tuberculin and lepromin tests, in tuberculoid lesions and in the epidermis and dermis during type I and II reactions. In multibacillary lesions, however, the number of LCs was consistently low in comparison with other groups[74]. Hunger et.al.[76] and Seiling et.al.[93] also concluded similar results.

4 out of 38 cases in the present study gave positivity for both the markers. 3 were BT and 1 was IL case. As already mentioned, Indeterminate leprosy is an early stage of leprosy evolution where the histological and immunological responses have not evolved completely [58,65]. Limitation of the present study however is the sample size (low specifically owing to the COVID pandemic hampering non-covid patient visit to the hospital). Owing to lesser number of cases under evaluation, the results cannot be applied in general.



A- Strong S100 staining of macrophages within the dermis of skin in a case of Lepromatous Leprosy.
B- S100 negative in a case of Tuberculoid leprosy
C- Strong CD1a staining of Dendritic cells in the epidermis in a case of Tuberculoid Leprosy.
D- CD1a negative staining in a case of Lepromatous Leprosy(no immune activedendritic cells in the epidermis)

Illustration-1

CONCLUSION:

A retrospective and prospective study was conducted to determine the spectrum of leprosy disease in 38 cases in a tertiary care centre.

Clinical assessment as well as histopathological (gold standard) diagnosis was given. Immunohistochemistry using S100 and CD1a was performed. S100 expression was studied over macrophages and CD1a over langerhan cells.

S100 predominance was seen in lepromatous polar leprosy whereas CD1a expression was intensely seen in the tuberculoid leprosy cases. Borderline tuberculoid and Indeterminate leprosy cases gave variable results.

CD1a staining LCs basically represent the cellular immunity of the host which is competent towards tuberculoid spectrum. S100 stains basal melanocytes (internal control), Schwann cells(around nerve bundles) and macrophages.

Our study was limited clinic-pathological diagnosis and did not deal with the treatment outcome. To established the prognostic significance of the IHC markers in leprosy, more detailed large cohort multi-centric clinical trials should be advised.

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