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Original Research Paper

To Study the Expression of S100 and Factor XIIIa as immune markers in leprosy

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ABSTRACT:

Background: Macrophages are critical for the lysis of bacteria in the defence against Mycobacterium leprae. However, cytokines from lymphocytes, such as interleukin 2 and gamma interferon, are also necessary for the effective killing of the organisms. In this study, S100 and factor XIIIa were assessed by Immunohistochemistry on the cutaneous lesions of 45 leprosy cases. **Aim**: To study the role of S100 and Factor XIIIa as immune markers in leprosy. **Study design**: Cross sectional study. **Methods**: Skin biopsies of clinically suspected leprosy were stained for histopathological examination, and Immunohistochemistry examination was carried out for S100 and factor XIIIa. **Result:** S100 expression was studied over macrophages and factor XIIIa on dermal dendrocytes. S100 positivity was seen in the lepromatous leprosy spectrum, while Factor XIIIa was observed in tuberculoid, with few indeterminant and histoid cases showing immunoreactivity. **Conclusions**: IHC markers can be used as an auxiliary technique to support histopathological diagnosis and aid in early and more specific diagnosis in early leprosy cases, assessing the host's immune status. Therefore, it can guide early initiation of treatment, modification of regimes and follow-up schedules.

Keywords:* *Leprosy, Immunity, S100, Factor XIIIa

INTRODUCTION:

Leprosy is a chronic infection with high infectivity and low pathogenicity caused by Mycobacterium leprae. This disease is called Hansen's after the Norwegian scientist Gerhard-Henrik Armauer Hansen, who found Mycobacterium leprae in 1873 (1). The patient's immunological status influences how the disease manifests itself in the course of leprosy infections. After infection, progression to one of the following four forms of leprosy manifests depending on the immune response of the patient: lepromatous, tuberculoid, borderline or indeterminate (2). Histoid leprosy is a rare multibacillary form (3).

Recognising these immunological mechanisms is essential for the management and control of the disease because the various clinical signs and symptoms of leprosy are linked to the host's immune response to bacilli (4). Leprosy's histopathological study aids in making a conclusive diagnosis, which can be challenging clinically. Immunohistochemistry, which uses specialised antibodies, has developed into a valuable tool for research on the immunopathology of leprosy. Leprosy lesions are further categorised using immunohistochemical markers, which can help in tracking the progression of the disease and the effectiveness of treatment. S100 proteins and Factor XIIIa have emerged as potential candidates among the various markers used to shed light on the intricate immunological interactions in leprosy lesions (5).

Macrophages belonging to the tuberculoid spectrum phagocytose the bacilli and then kill them, so hindering their proliferation. The lepromatous spectrum bacilli are unable to destroy the bacilli, resulting in its multiplication. Though the disease has been traditionally defined along a Th1/Th2 spectrum, where the Th1 pole corresponds to the paucibacillary

spectrum and the Th2 to the multibacillary spectrum, both innate and acquired immune responses are involved (6). Blood monocytes that migrate into the skin in response to chemotactic stimuli are the source of the phagocytic cells found in the skin. They become epithelioid cells as a result of active macrophage cells clumping together, growing, and having more eosinophilic cytoplasm. The antigen-presenting cells, known as dendritic cells (DC), phagocytose the bacteria. In the skin, DC is expressed by epidermal Langerhans cells and dermal dendrocytes (2). Dermal dendrocytes are histogenetically related to macrophages and can be identified by their immunophenotype. Utilising immunohistochemical methods, this differentiation is achieved by showcasing traits that all mononuclear phagocytic system cells have in common. These cells seem to be part of an immunologically competent native system of the dermis. A newly identified subset of dermal dendrocytes known as Factor XIII+ can be identified by the expression of coagulation Factor XIIIa in their cytoplasm. These cells have a dendritic morphology and are numerous in the papillary dermis. Histoid leprosy is lepromatous leprosy characterised by nodules formed by fusiform histiocytic cell whorls and fascicles containing several Mycobacterium. This lesion is typically present in lepromatous leprosy that has relapsed (7, 8). Dendritic cells that are S100 negative, phagocytic, and positive for the coagulation protein Factor XIIIa have been discovered in the papillary dermis (9-10).

This study's primary goal was to observe the role of macrophages and dendritic cells in the pathogenesis of leprosy and to clarify the potential diagnostic and prognostic value of S100 and Factor XIIIa in leprosy lesions while advancing our knowledge of the immunological aspects of this complex disease.

MATERIAL AND METHODS:

A sample size of 45 cases with skin biopsies or specimens received in the pathology lab of patients diagnosed with leprosy recruited in a tertiary care hospital.

According to the Inclusion Criteria: All skin biopsies of suspected leprosy lesions. Participants of all ages and genders diagnosed with different forms of leprosy (e.g., tuberculoid, lepromatous) were included. Inadequate, non-representative samples and Autolysed samples were excluded. All the skin biopsies of leprosy with respect to prospective cases were collected in 10% formalin. In cases involving a review of past work, pertinent paraffin-embedded tissue blocks were in the department's archive and relevant case information was recorded using the test request form.

Fresh slides were prepared and stained for examination.

Histopathological examination was carried out, and relevant parameters were assessed.

Paraffin tissue sections were prepared on precoated slides for immunohistochemistry (IHC). IHC was carried out by standard protocols.

Approval from Institutional Ethical committee was taken.

Statistical Analysis:

The data obtained was coded and then entered into Excel spreadsheet. Data analysis was conducted using SPSS, version 25.0, the statistical tool for the social sciences. A combination of tabular and graphic formats was used to present the results. Chi-square test was used to compare the percentages and frequencies of the qualitative data. Mean/Median and SD/IQR were the quantitative data presentation methods used based on the sample distribution. Random t-test with Mann-Whitney U For the quantitative data variables, the U test was utilized to compare the two separate groups. <0.05 P-value is considered significant

OBSERVATION AND RESULTS:

This study aimed to provide insight into the function of Factor XIIIa and S100 proteins in leprosy differentiation.

The histopathological diagnoses of the 45 leprosy patients are detailed in Table 1. The most common diagnosis was lepromatous leprosy, observed in 18 patients, accounting for 40.0% of the sample.

The distribution of S100 protein expression in macrophages across different histopathological diagnoses of leprosy is detailed in Table 2 and reveals significant findings (p-value < 0.001). Among the 45 patients, those diagnosed with lepromatous leprosy exhibited a high positive expression of S100, with 15 out of 18 cases (83.3%) testing positive. In contrast, patients with other types of leprosy showed no S100 expression in macrophages. These results highlight a strong association between lepromatous leprosy and S100 positivity, suggesting the potential role of S100 protein as a marker for this particular type of leprosy.

The distribution of Factor XIIIa expression in dermal dendrocytes across different histopathological diagnoses of leprosy is detailed in Table 3 and reveals significant associations (p-value < 0.001).

Among the 45 patients, those with histoid leprosy had 3 out of 4 cases (75%) positive for Factor XIIIa expression.

Indeterminate leprosy patients showed an equal distribution, with 3 cases positive and 3 negative. Tuberculoid leprosy showed a high positive expression of Factor XIIIa, with 11 out of 16 cases (68.8%) testing positive. In contrast, all 18 cases of lepromatous leprosy were negative for Factor XIIIa expression. The single case of mid borderline leprosy also tested negative. These results underscore a significant correlation between Factor XIIIa expression

and specific types of leprosy, particularly histoid and tuberculoid.

TABLES:

Table 1: Histopathological diagnosis of patients:

HPE diagnosis	Frequency	Percent
Histoid Leprosy	4	8.9
Indeterminate Leprosy	6	13.3
Lepromatous Leprosy	18	40.0
Mid borderline Leprosy	1	2.2
Tuberculoid leprosy	16	35.6
Total	45	100.0

Table 2: Distribution of patients on histopathological diagnosis and s100 expression in macrophages

		S100_in_macrophages		Total	p-value
		Negative	Positive		
HPE diagnosis	Histoid Leprosy	4	0	4	<0.001
	Indeterminate Leprosy	6	0	6	
	Lepromatous Leprosy	3	15	18	
	Mid borderline Leprosy	1	0	1	
	Tuberculoid leprosy	16	0	16	
Total		30	15	45	

Table 3: Distribution of patients on histopathological diagnosis and Factor XIIIa expression in dermal dendrocytes

		Factor XIIIa in dermal dendrocytes		Total	p-value
		Negative	Positive		
HPE diagnosis	Histoid Leprosy	1	3	4	<0.001
	Indeterminate Leprosy	3	3	6	
	Lepromatous Leprosy	18	0	18	
	Mid borderline Leprosy	1	0	1	
	Tuberculoid leprosy	5	11	16	
Total		28	17	45	

DISCUSSION:

India accounted for more than 50% of all leprosy patients worldwide more than three decades ago, when the disease was widespread. India still accounts for a significant portion of the global leprosy population—58.8%—even though an effective multidrug therapy (MDT) has been available and in use for more than 30 years and the disease has been eliminated (1 case/10,000 population size, as defined by the World Health Organization [WHO]) since 2002 (11-12).

Understanding molecular markers and how they relate to different histopathological characteristics is essential to improving leprosy diagnosis and treatment. Because of the Mycobacterium leprae, leprosy is a long-term infectious disease (13).

Several cellular processes, including inflammation and immunological responses, are regulated by S100 proteins and other members of the calcium-binding protein family (14). Their differential expression in macrophages is associated with the pathophysiology of various inflammatory and infectious conditions, which gives them potential as diagnostic biomarkers for leprosy (15).

In a similar vein, the role of Factor XIIIa, a transglutaminase enzyme primarily expressed by dermal dendrocytes, in tissue repair and fibrosis has come to light (16, 17). In light of this, the current study looked into the expression patterns of two important proteins, S100 and Factor XIIIa, in leprosy patients, highlighting their potential as diagnostic tools and links to various disease phenotypes.

The significance of immunohistochemical markers, Factor XIIIa and S100 protein, in clarifying the immunopathogenesis of leprosy and assisting in its classification has been brought to light by recent studies. This study examined the complex roles that Factor XIIIa and S100 proteins play in leprosy differentiation, providing insight into their correlation and diagnostic value in different forms of leprosy. The goal of the thorough cross-sectional analysis involving 45 patients was to improve our knowledge of the expression patterns of these proteins and how they relate to various histopathological diagnoses.

Histopathological diagnoses revealed that Lepromatous leprosy was the most prevalent type in our study, with tuberculoid leprosy trailing closely behind. The study participants' heterogeneous distribution of leprosy types is indicative of the range of clinical presentations and immune reactions that Mycobacterium leprae infection can cause.

The current study's examination of the expression of the S100 protein yielded important information regarding its correlation with various histopathological forms of leprosy, with significant therapeutic and diagnostic implications. The results of the analysis showed a strong relationship between lepromatous leprosy and S100 protein expression, with the vast majority of cases showing positive staining in macrophages.

This startling discovery raises the possibility that the S100 protein may serve as a diagnostic marker for lepromatous leprosy, setting it apart from other types of the illness (18). S100 positivity and lepromatous leprosy are strongly correlated, which highlights the unique immunopathological characteristics of this phenotype, which include a pronounced humoral immune response and widespread bacillary proliferation.

Numerous investigations into the expression patterns and diagnostic applications of S100 protein in a range of infectious and inflammatory diseases have yielded important new information about this protein's potential as a biomarker. For example, S100 protein expression in macrophages has been linked to disease severity and response to treatment in dermatological disorders such as psoriasis (19).

In a similar study conducted by Cuevos Santos et al, all cases of lepromatous spectrum showed intense immune positivity for S100 (5). It suggested the macrophages are derived from monocytes in blood and then divided into S100 positive and S100 negative populations. They proposed a transition of this sub-population from one pole to another. Mohanraj et al. conducted a study using S100 staining to assess dendritic cells in leprosy in which he observed membranous positivity in lepromatous cases (18).

Investigating the expression of Factor XIIIa in relation to leprosy provides fresh perspectives on the immunopathogenesis of this intricate illness and helps to discover new biomarkers for enhanced diagnosis and treatment approaches.

The current study's analysis of Factor XIIIa expression shed light on the immunopathogenic mechanisms underlying leprosy and its potential diagnostic significance by revealing its correlation with different histopathological types of the disease. Significant differences in Factor XIIIa expression were found amongst leprosy phenotypes, with histoid, tuberculoid, and lepromatous forms exhibiting the most notable variations.

Among the histopathological types we studied, histoid leprosy showed a notable positive rate for Factor XIIIa expression in dermal dendrocytes, with 75% of cases showing positive staining.

Three histoid leprosy patients' cutaneous lesions tested strongly positive for Factor XIIIa in a study by Cuevas-Santos J et al., indicating the role of Factor XIIIa-positive dermal dendritic cells in histoid leprosy (5).

On the other hand, in every case studied, lepromatous leprosy consistently displayed negative expression of Factor XIIIa in dermal dendrocytes. This fascinating discovery points to a unique immunological profile linked to lepromatous leprosy, which may include altered wound healing processes and decreased Factor XIIIa activity. The lack of Factor XIIIa expression in lepromatous leprosy highlights the need for more research to clarify the underlying mechanisms governing Factor XIIIa regulation in leprosy and raises concerns about its role in the immunopathogenesis of this form of the disease.

In addition, a significant positive rate for Factor XIIIa expression was observed in tuberculoid leprosy, with 68.8% of cases demonstrating positive staining in dermal dendrocytes. This result points to a possible correlation between the immunological profile of tuberculoid leprosy, which is marked by the development of granulomas and a robust cell-mediated immune response, and the expression of Factor XIIIa. Further research into the potential diagnostic and prognostic utility of Factor XIIIa positivity in tuberculoid leprosy, in differentiating leprosy phenotypes and directing therapeutic interventions, is warranted given the observed correlation between it and the disease.

CONCLUSION:

The collective findings from the studies examining S100 and Factor XIIIa expression in leprosy highlight the intricate interplay between immunological markers and disease phenotypes. S100 protein expression emerged as a potential diagnostic marker, particularly in distinguishing lepromatous leprosy, while Factor XIIIa expression exhibited variability across different forms of the disease, suggesting its involvement in the immunopathogenesis of leprosy. These results underscore the heterogeneity of immune responses and immunopathogenic mechanisms underlying different leprosy phenotypes, with S100 and Factor XIIIa expression patterns reflecting variations in the host immune response and disease progression. Further research into the functional roles of these markers may provide valuable insights into the development of targeted diagnostic and therapeutic strategies for this complex infectious disease.

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