S100 Immunohistochemistry can Identify Differences in Peripheral Nerve Damage Patterns in Paucibaciler and Multibaciler Types of Morbus Hansen

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Received : 12-04-2023
Accepted : 26-04-2023
Published : 31-05-2025

ABSTRACT

Background
Morbus Hansen (MH) is a granulomatous infectious disease of the skin caused by *Mycobacterium leprae*, which induces skin lesions and peripheral nerve injuries that can cause physical disabilities. The basis of the diagnosis of MH is based on classic clinical signs, characteristic histopathological findings and demonstration of acid-fast bacilli in skin biopsies. However, determining neurovascular involvement in a granulomatous reaction is challenging in pathologic diagnosis. S100, a marker of pheripheral nerve, may aid MH diagnosis. The aimed of this study was to determine different pattern of S100 immunostaining in paucibacillary and multibacillary type MH.

Methods
This research was an analytic cross-sectional study. Sample was MH patients who underwent a skin biopsy and was examined for histopathology by routine H&E staining and *Ziehl Neelsen* (ZN) staining at the Anatomical Pathology Laboratory, Faculty of Medicine, Udayana University/Prof.Dr. I.G.N.G Ngoerah hospital, Denpasar from 2017 to 2022. Clinical information, histopathological examination results and ZN stain data were obtained. Pattern of neurovascular damage was determined by S100 immunohistochemistry and catagorized into intact infiltrated pattern and reduced/fragmented infiltrated patterns. The association between MH type and S100 staining pattern was analyzed by Chi-square test with a significance value of $p<0.05$.

Results
The results showed that the immunostaining pattern in paucibaciler type MH was mostly reduced/fragmented infiltrated pattern, meanwhile in multibacillary type MH was mostly intact infiltrated pattern. There was a significant different S100 immunostaining pattern in Paucibaciler and Multibaciler MH ($p=0.001$).

Conclusion
S100 immunostaining could identify differences in immunostaining patterns in cases of paucibacillary and multibacillary MH types. S100 may used as aditional testing to determine neurovascular involvement.

Keywords: Morbus Hansen, S100, immunohistochemistry.

P-ISSN 0215-7284
e-ISSN 25279106
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BACKGROUND

Morbus hansen (MH) is one of the oldest diseases known by human, caused by *Mycobacterium leprae*, induced skin lesions and peripheral nerve injuries that could cause physical disabilities. Diagnosis of MH is based on classic clinical signs, characteristic histopathological findings and demonstration of acid-fast bacilli in skin biopsies. However, the difficulty in histopathological diagnosis was a challenge to prevent delays in diagnosis and the occurrence of defects.[1-4]

The global prevalence of MH disease was around 224 000 in early 2007. Estimates of the global prevalence of this disease were around 210 000 in 2015, indicating the number of cases worldwide has been relatively static over the last decade. These data indicate that WHO targets for disease elimination have not been resolved. Morbus Hansen continued to be a major cause of infection-related morbidity in many countries, particularly the 136 MH endemic countries, despite advances in effective multidrug treatment over the last three decades.[3]

The national target were set for the number of new cases of MH in 2017 is less than 5/100.000 population. The number of new MH cases found in Indonesia in 2017 was 6.08 cases / 100.000 population. Meanwhile, the New Case Detection Rate for MH for Bali Province in 2017 was 1.84/100.000 population, higher than 2016 of 1.047/100.000 population. This situation showed a relatively static condition. This could be seen from the number of new MH cases discovered, which ranged from 1-2 cases per 100,000 from 2012 to 2017.[5,6]

There were several classifications, including Ridley-Jopling and WHO. This classification was made because of the variation in manifestations, course of the disease, prognosis and management of paucibacillary and multibacillary MH. Standard treatment for multibacillary MH consists of Rifampicin, Clofazimine, and Dapsone with duration of treatment is 12 months. Meanwhile, standard treatment for paucibacillary MH consists of Rifampicin and Dapsone with duration of treatment were 6 months. Multibacillary MH is more infectious disease, meanwhile nerve destruction is more often in paucibacillary MH.[6,7]

Based on the pathophysiology, the bacteriological-immunological response in paucibacillary-type MH would stimulate pro-inflammatory cytokines that trigger a cell-mediated immune response that causes damage to the surrounding nerve structures and adnexal glands, whereas in multibacillary-type MH it is mediated by humoral immunity which has more role in neutralizing toxins, as well as phagocytosis, without further destruction.[2,7]

Routine histopathological examination using hematoxylin & eosin (HE) stains was not always able to determine and show nerve branches within the granuloma; while S100 immunostaining, Schwann cell marker, could be used to describe nerve involvement in paucibacillary and multibacillary MH types.[2,7,8]

It is important to make a definite diagnosis because paucibacillary and multi-bacillary MH have different course of disease, management, and prognosis. The aimed of this study was to determine different pattern of S100 immunostaining in paucibacillary and multi-bacillary type MH.

METHODS

This research was an analytic observational study using cross-sectional analytic study design. This research has received approval from the Research Ethics Committee of the Faculty of Medicine, Udayana University No. 46/UN14.2.2.VII.14/LT/2023.

The sample of this study was 40 total MH patients cases who underwent skin biopsy and were examined for histopathology with routine H&E staining and *Ziehl Neelsen* (ZN) histochemical staining at the Anatomical Pathology Laboratory, Faculty of Medicine, Udayana University/Prof. Dr. I.G.N.G Ngoerah hospital, Denpasar from 1 January 2017 to 31 December 2022. The inclusion criteria was: paraffin block preparations from skin biopsy material with a histopathological diagnosis of MH of various types (PB and MB), accompanied by the availability of ZN staining data; and the paraffin block was in good condition and still contained tissue with adequate MH skin lesions for resection and immunohistochemical examination. The exclusion criteria was cases with diagnosis indeterminate type MH. Evaluation of H&E and ZN slides were done by DC and HS.

The immunohistochemistry was done with Dako-Bondmax immunostainer using a polymer-based detection system. The antibody used was polyclonal antibody S100 ready to use PA0900 (Novocastra, UK). Heat antigen retrieval was done with Tris-EDTA at pH 9, in 100^0C, for 15 minutes.

Interpretation of S100 expression using an Olympus CX22 binocular light microscope. S100 staining pattern was categorized into: (1) Intact and infiltrated-Continuously and closely stained nerve fragments can be observed along with visible mixed inflammatory cells around it,

Maj Patol Indones 2025; 34(2): 900-905
DOI: 10.55816/mpi.v34i2.605

P-ISSN 0215-7284
e-ISSN 25279106
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(2) Reduced/Fragmented and infiltrated-Discontinues (fragmented) staining pattern and/or Very few visible stained nerve branches (nucleus and cytoplasm), which are separated and infiltrated by an inflammatory cell infiltrate.[9] Evaluation of S100 immunostaining was done by DC and NPS.

Data was tested by Chi-square analysis using SPSS (Statistical Package for the Social Sciences) 25.0 for Windows showed a statistically significant difference in the S100 immunostaining pattern in PB and MB subtypes (p=0.001). The significance was determined at α=0.05.

RESULTS

Forty cases that met inclusion and exclusion criteria were collected from histopathological archives at the Anatomical Pathology Laboratory of Prof. dr. I.G.N.G Ngoerah hospital, Denpasar, from January 2017 to December 2022. There were 22 cases with histopathological diagnosis paucibacillary type MH and 18 cases of multibacillary type MH. The highest frequency of occurrence of MH was in the age range 40-49 years (Figure 1).

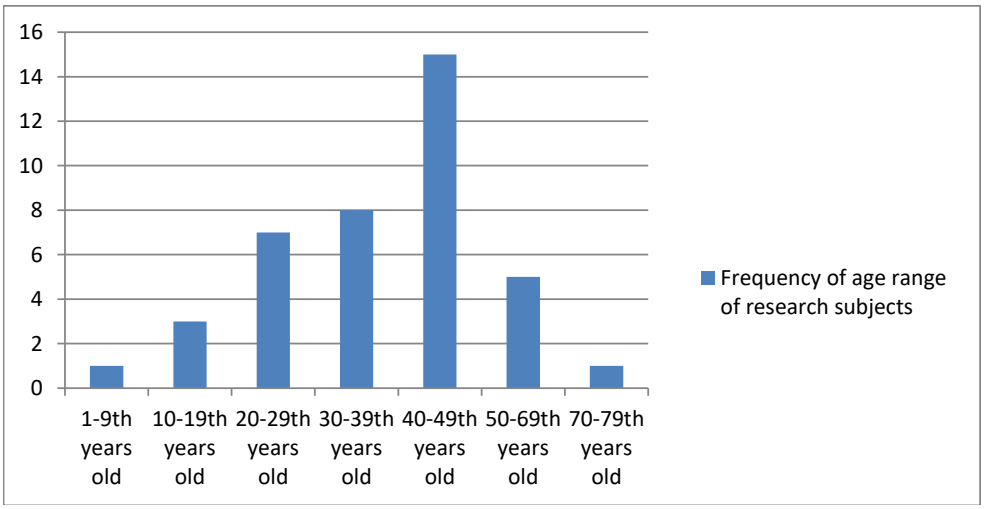

Figure 1. Distribution of patients by age groups.

Sex distribution in both type of MH is different. In male MH patients, PB type occurence was 2 times more common than MB type. In female patients, there was similar occurence of PB and MB subtype MH. Meanwhile, according to age group, in patients under 50 year old, proportion of PB and MB cases were equal. But, in patients more than 50 years old, majority of patients with PB subtype (Table 1).

Table 1. Distribution of Morbus Hansen Patients based on sex and age characteristics.

Characteristics	Morbus Hansen		Total
	PB	MB	
Sex			
Male	16 (69.5%)	7 (30.5%)	23 (100%)
Female	9 (53 %)	8 (47 %)	17 (100%)
Age			
<50 years old	17 (50%)	17 (50%)	34 (100%)
≥50 years old	5 (83.3%)	1 (11.7%)	6 (100%)

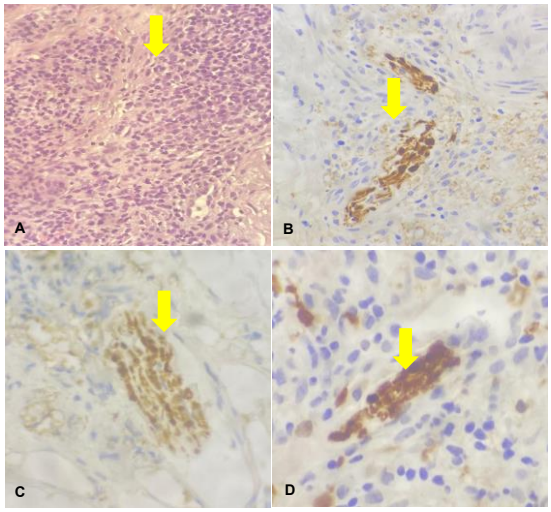

Figure 2. A. Granulomatous pattern in PB type MH observed by H&E stained with no obvious peripheral nerve involvement. B. Peripheral nerves involvement observed by S100 immunostaining (arrows) of the skin tissue biopsy. C. Fragmented immunostaining pattern and infiltrated by inflammatory cells. D. Intact immunostaining pattern and infiltrated by inflammatory cells. (H&E, S100, 400 times).

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P-ISSN 0215-7284
e-ISSN 25279106
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In this study it was shown that there was a significant difference between the pattern of S100 immunohistochemistry in 22 patients (55%) of MH paucibacillary type and 18 patients (45%). From all PB type MH cases, 21 of 22 patients showed reduced/fragmented and infiltrated pattern and 1 of 22 ptients showed intact infiltrated staining pattern. Whereas in MH type MB, 14 of 18 patients showed an intact infiltrated staining pattern and 4 other patients showed a fragmented infiltrated staining pattern. Chi-square analysis showed a statistically significant difference in the S100 immunostaining pattern in PB and MB subtypes (p=0.001).

Table 2. S100 immunostaining pattern in Paucybacillary and Multibacillary type Morbus Hansen.

Staining Pattern	MH type		Total, n=40	p-value
	PB, n=22	MB, n=18		
Reduced/fragmen-ted and infiltrated	21 (52.5%)	4 (10%)	25	0.001*
Intact and infiltrated	1 (2.5%)	14 (35%)	15	

*Statistically significant if p<0,05.

In addition, S100 immunostaining patterns for MH type based on Ridley-Jopling classification was also determined (Table 3).

Tabel 3. Frequency of S100 immunostaining pattern for MH type based on Ridley-Jopling Classification.

Staining Pattern	MH					Total
	TT	BT	BB	BL	LL	
Reduced-fragmented infiltrated	7	15	0	1	2	25
Intact infiltrated	0	1	2	11	1	15
Total	7	15	2	13	3	40

Reduced/fragmented and infiltrated staining patterns were found in all TT-type MH cases (7 of 7 patients) and majority of BT-type MH cases (15 of 16 patients). It was reduced/fragmented pattern was found in most of PB-type MH. Meanwhile, in the intact infiltrated staining pattern, most of the cases with MB type MH (2 patients from BB type MH, 11 patients from BL type MH, and 1 sample from LL type MH).

DISCUSSION

Diagnosis of MH requires classic clinical signs, characteristic histopathological findings and demonstration of acid-fast bacilli in skin biopsy tissue. Diagnostic accuracy of MH is useful for the benefit of the leprosy management program in the WHO classification, namely paucibaciler (PB) and multibaciler (MB) which are different.[10]

Difficulty in histopathological diagnosis is a challenge to prevent delays in diagnosis and the occurrence of defects, as well as prevent inaccuracy of management. Skin biopsy tissue showing a granulomatous inflammatory reaction can complicate the assessment of neurovascular damage or the involvement. Also, MH tuberculoid and indeterminate forms, the detection rate of *M. leprae bacilli* is still low or difficult. Histological evidence of active destruction of peripheral nerves in skin biopsy tissue by granulomatous inflammation is widely accepted as a feature of MH lesions. However, identifying nerve remnants among the granulomas becomes difficult in some cases, and morphological similarities to granulomatous dermatoses, such as lupus vulgaris, secondary syphilis, and sarcoidosis will add to the problem.[9,11,12]

S100 immunohistochemistry is a marker that is widely known to have a role as a specific antibody specifically labeling glial cells and Schwann cells, so it is considered to be sensitive in assessing nerve damage and reliable in visualizing peripheral nerves, especially in granuloma reactions which make it difficult to assess neurovascular involvement, especially in MH type PB and indeterminate forms.

Without visualization of *Mycobacterium Leprae* bacteria on ZN histochemical staining, and it is difficult to assess neurovascular appearance in the granuloma inflammatory reaction, this requires additional S100 immunohistochemistry to assess peripheral nerve damage between granuloma tissue reactions which show a fragmented pattern of staining pattern in MH PB type.[9,11,12]

Based on the pathophysiology, the bacteriological-immunological response in paucibaciler-type MH will stimulate pro-inflammatory cytokines mediated by Th-1 which will trigger a cell-mediated immune response that causes damage to the surrounding nerve structures and adnexal glands, whereas in contrast, in multibaciler-type MH, it is mediated

Maj Patol Indones 2025; 34(2): 900-905
DOI: 10.55816/mpi.v34i2.605

by humoral immunity. mediated by Th-2 play a greater role in neutralizing toxins, as well as opsonization of germs, without further destruction. Therefore, PB type MH can provide an overview of the S-100 reduced – fragmented and infiltrated IHC pattern depending on the clinical spectrum of TT or BT. Likewise in MH type MB, due to less destructive damage to the peripheral nerves, it will give an intact infiltrated pattern.[2,7]

In this study it was shown that there was a significant difference between the pattern of S100 immunohistochemistry in PB and MB type MH. Almost all of PB type MH showing reduced-fragmented and infiltrated staining pattern, only 1 patient showed intact infiltrated pattern. Whereas in MH type MB, majority of the patients showed an intact and infiltrated staining pattern. As previously explained, PB type MH which generally occurs in patients with better immunity can give a more severe inflammatory reaction, will stimulate pro-inflammatory cytokines that trigger a cell-mediated immune response that causes damage to nerve and adnexal gland structures, so that a pattern is determined. S100 immunostaining of peripheral nerves in PB-type MH due to Mycobacterium leprae infection was a reduced-fragmented-infiltrated pattern. Meanwhile, the MB type MH is expected to provide an intact infiltrated stain pattern.[2,7]

In the previous study, which was conducted by Shenoy and Nair (2018), also obtained results indicating a difference in the pattern of staining between MH type PB and type MB. In the study they conducted, 39 patients (78%) were found with a reduced / fragmented - infiltrated pattern from 50 patients of MH PB type. Likewise, in a study conducted by Manjunath et al., (2021), similar results were obtained that the S100 immunohistochemical stain pattern in skin biopsy tissue with a diagnosis of MH PB type was in the form of a fragmented – infiltrated pattern in 46 patients (79%) of a total of 58 patients which they studied.[9,11]

Based on the results of the current study and research that has been carried out in previous studies by other researchers, similar results were obtained that the pattern of S100 immunostaining in skin biopsy tissue with a diagnosis of PB type MH showed mostly fragmented-infiltrated patterns and S100 immunostaining pattern in tissue biopsies. skin with the diagnosis of MH type MB, almost all of them show an Intact – infiltrated pattern. Therefore, for a more accurate diagnosis of MH the S100 immunohistochemistry will be very useful.

In this study, based on Ridley-Jopling classification, all TT type MH showed reduced/fragmented and infiltration immunostaining pattern. In TT type MH where tuberculoid granuloma can easily found meanwhile bacterial findings often negative, S100 immunostaining will help to detect peripheral nerve destruction and inflammation.

CONCLUSION

S100 immunostaining could identify differences in immunostaining patterns in cases of paucibacillary and multibacillary MH types. S100 may used as aditional testing to determine neurovascular involvement.

To increase the consistency of the results of this study, further research can also be carried out using prospective research methods and involving multicenters to support the results of this study.

Research Ethics

This research has received approval from the Research Ethics Committee of the Faculty of Medicine, Udayana University with no. 46/UN14.2.2.VII.14/LT/2023.

Conflict of Interest

The author declares that there is no conflict of interest regarding the publication of this research.

Funding

This research did not receive grants from the government or other private sectors.

Author Contributions

Dandy Citra has contributed to the preparation of research concepts and designs, literature studies, histopathology and immunohistochemistry interpretation, collection and analysis of data, statistical analysis, and manuscripts drafting.

Ni Putu Sriwidyani has contributed to the preparation of research concepts and designs, immunohistochemistry interpretation, manuscript review.

Herman Saputra has contributed in the preparation of research concepts and designs, literature studies, histopathology interpretation.

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P-ISSN 0215-7284
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