

Mast Cells in Leprosy Patients with Reversal Reactions

Tip 1 Reaksiyon Pozitif Lepra Hastalarında Mast Hücreleri

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Abstract

Background: Reversal reaction (RR) is an acute reaction on chronic episode which often contributes to withdrawal of treatment and disability in leprosy patients. Previous study showed that mast cells in reactive tuberculoid lesions biopsy material had lower density than non reactive tuberculoid lesions. Granulomatous lesions in leprosy patients with high mast cells density were more likely to progress into severe reversal reaction.

Objective: To examine mast cells level in reversal reaction patients (RR) and non-reactional leprosy patients (non-RR)

Methods: Fifty-six leprosy patients consist of 28 with reversal reactions and 28 non-reactional leprosy were assessed for mast cells levels by immunohistochemistry staining. The mast cell numbers in both groups were analyzed using Independent T-test.

Results: Mast cells were increased in the reversal group ($p < 0.05$).

Conclusion: Mast cells level is associated with reversal reaction of leprosy.

Keywords: Leprosy, reversal reaction, mast cell

Öz

Giriş: Tip 1 reaksiyon, genel olarak lepra hastalarında hareket kısıtlılığı ve tedavinin kesilmesi ile ilişkili olan, kronik lepra hastalığının akut bir reaksiyonudur. Çalışmalar, reaktif tüberküloid lezyonlardan alınan biopsi materyallerinde, reaktif olmayan tüberküloid lezyonlarındakine göre daha az mast hücresi olduğunu göstermiştir. Lepra hastalarının granülomatöz lezyonlarındanda dansitesi çok yüksek olarak saptanan mast hücreleri, muhtemelen Tip 1 reaksiyona neden olmaktadır.

Amaç: Tip 1 reaksiyon gösteren hastalar ile göstermeyen hastaların mast hücresi düzeylerini araştırmak.

Yöntemler: Reaksiyonu olan 28 hasta ile reaksiyon göstermeyen diğer 28 hasta, immünohistokimya boyaması ile saptanan mast hücreleri açısından irdelendi. Her iki gruptaki mast hücresi sayıları, Student-t testi ile karşılaştırıldı.

Bulgular: Reaksiyon gösteren grupta mast hücreleri istatistiksel olarak anlamlı düzeyde daha fazla idi ($p < 0.05$).

Sonuç: Lepra hastalarında, mast hücreleri tip 1 reaksiyon ile ilişkilidir.

Anahtar Kelimeler: Lepra, Tip 1 reaksiyon, mast hücresi

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Introduction

Leprosy is a curable chronic infectious disease which can cause severe morbidity associated with disability. Leprosy is caused by intracellular obligate bacteria, *Mycobacterium leprae*. *M. leprae* infects peripheral nerve, skin, oral mucosa, upper respiratory airway, reticuloendothelial system, eyes, muscles, bone, testicles, and all of human organs other than central nervous system. Leprosy reactions are acute exacerbations that manifest as activation of constitutional symptoms and/or new skin efflorescence. There are two types of leprosy reactions: type 1 (Reversal Reaction/RR) and type 2 (Erythema Nodosum Leprosum/ENL).^[1]

Reversal reaction (RR) is type IV hypersensitivity reaction which frequently occurs in borderline type leprosy as a result of cellular immune response to *M. leprae* antigen and characterized as an acute inflammation of the former skin lesion. Approximately 95% of RRs occur simultaneously with diagnosis confirmation or during multi-drug treatment (MDT). RR commonly appears on the first six-month treatment, particularly in the borderline tuberculoid (BT) and borderline borderline (BB) leprosy. It can also be found in borderline lepromatous (BL) leprosy with longer interval of MDT. Clinical manifestation of RR includes abrupt increase in number and more active lesions with/without ulceration, edema, neuritis, and permanent nerve damage. Bacterial index (BI) is frequently negative or remarkably decreased in RR patients. Leprosy patients commonly complain about skin lesion enlargement which is aesthetically disturbing. This causes treatment withdrawal since patients consider it as a treatment failure.^[1,2]

Mast cell is a constant cellular component on dermis, lamina propria of mucous, serous, and connective tissues.^[3] In the tissue damage, mast cell degranulation and release of inflammation mediator cause increase in vascular permeability, local edema, leukocyte activation, tissue destruction, and eventually skin lesion as a result of inflammation response. Chowdory and Gosh showed that mast cell in the reactive tuberculoid lesion biopsy had lower density than non-reactive tuberculoid lesions.^[4] Several studies also found that granulomatous lesions in leprosy patient with high density level of mast cells were more vulnerable to become severe RRs.^[4,5] There were no studies which explain roleplay of mast cells in immuno-inflammatory processes in RR and non-RR leprosy patients.

Materials and Methods

Research design in this study was analytic observational with cross sectional assessment. Subjects were multi-bacillary

leprosy patients treated at the Donoharjo Hospital, Jepara. Subject selection has been done by consecutive sampling with double-blind method. Participants were multi-bacillary leprosy patients who met WHO criteria and aged between 20 and 60 years. Participants were divided into two groups: leprosy patients with RR, and leprosy patients without RR. Written informed consents were obtained from all participants. Exclusion criteria included pregnancy and other acute inflammatory diseases. Based on the sample calculations, total subjects in this study were 56 samples in two groups: leprosy patients with RR (n=28), and leprosy patients without RR (n=28). Mast cell levels were measured by immuno-histochemistry staining using monoclonal antibodies to Human Mast Tryptase antibody (Leica Biotech). It was visualized by DAB, and calculated by observing 20 fields of view in 100x magnifications.

The mast cell levels in both groups were respectively analyzed using independent samples T-test. The data were analyzed using normality and homogeneity test for age and occupation. Confounding factors in this study were controlled by randomization processes. The data were considered significant if $p < 0.05$ with confidence interval 95%. This study was approved by Ethics Committee of Faculty of Medicine, University of Airlangga.

Results

On the average, the age of studied participants, also number of men and women in control group were not much different from RR group (Table 1).

There was a significant increase ($p < 0.0001$) in the expression of mast cells in RR group (mean=20.86) compared with the non-RR (mean=9.29) (Table 2).

Table 1. Characteristics of subjects

		Non-RR	RR
	N	28	28
Age	Mean \pm standard deviation	41.00 \pm 8.26	42.43 \pm 9.13
Sex	Male	15 (53.6%)	12 (42.9%)
	Female	13 (46.4%)	16 (57.1%)

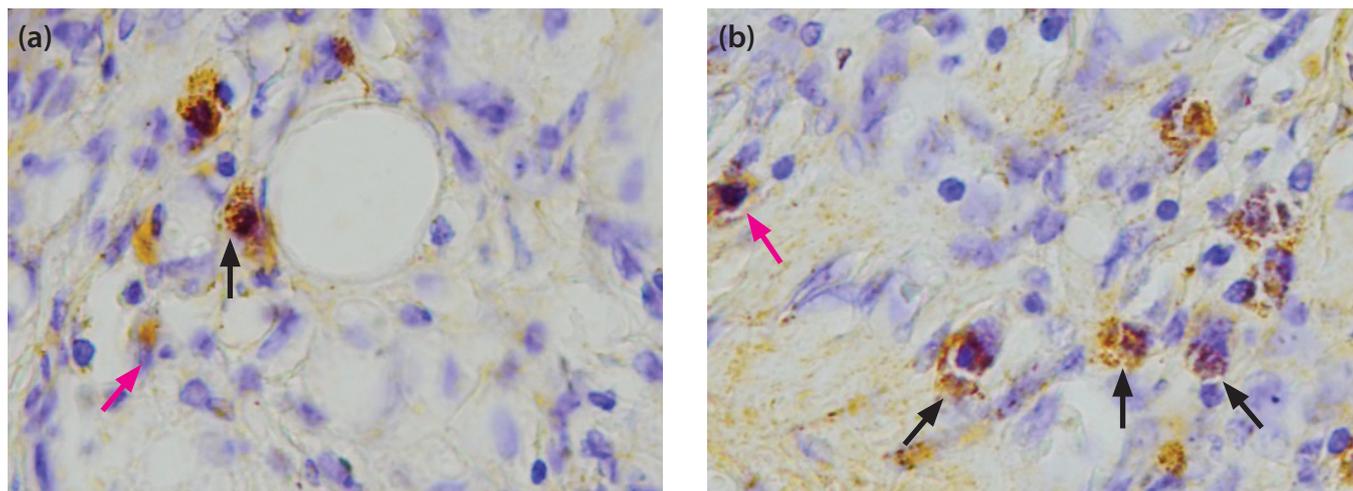


Figure 1. a, b. The figures show mast cells that give a positive reaction to anti-mast cells through immuno-histochemical staining (x400). Non-RR group (a); RR group. Black arrows indicate the double stained anti-mast cells. Red arrows indicate the mast cells that does not give positive reaction.

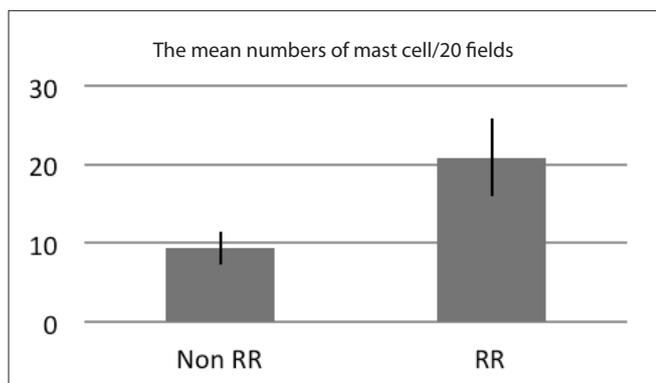


Figure 2. The histogram above shows increased expression of mast cells in RR group compared to those with non-RR.

Discussion

This study was conducted to explain the actual role of mast cells in leprosy RR. Theoretically, RR in terms of micro-organism destruction is a positive factor, as it occurs massively. However, it is clinically dissatisfying because of acute inflammatory reaction which is aesthetically disturbing.^[6]

RR is marked by delayed hypersensitivity to *M.leprae* antigen (Gell & Coombs type IV reaction), and an abrupt increase in immune cell response.^[6] There was an increase in dermis lymphocyte with the disappearance of normal granuloma structure that would decrease bacterial effectivity. In addition, Langhans giant cell could be observed in the next stage of RR.^[7,8] RR affects 20–30% of leprosy patients.^[6]

Table 2. Results of independent samples T-test

Groups	n	Mean ± sd	p
Non-RR	28	9.29 ± 2.07	0.000
RR	28	20.86 ± 4.92	

RR: Reversal Reaction

This research showed that there was a significant difference ($p < 0.05$) of mast cells in both groups. There was an increase in mast cell amount as well as its activity.

Previous studies examined the relationship between mast cells and lepra bacilli in the foot skin of immunosuppressed mice inoculated by leprosy bacilli. The study showed that there were changes in the structure and morphology of mast cells in which the structural changes were caused by direct infection of leprosy bacilli, whereas the morphological changes such as mastocytosis and massive degranulation were responses to the leprosy bacilli. Changes in density and massive degranulation is commonly found on mast cells in the skin, the affected nerves, muscles, and blood vessels. In human studies involving 118 untreated leprosy cases and 20 healthy individuals, there were a minimal number of mast cells in healthy individuals. The number of mast cells significantly increased in cases of leprosy ($p < 0.01$) and particularly greater in lepromatous leprosy (LL) type ($p < 0.05$). There is also an increase in degranulation and morphological changes in LL type. These changes can be caused by the

release of cytokines from T lymphocytes. In addition, protease release from mast cells may play a role like plasma proteins such as albumin to form a histamine release peptide that will further trigger the activation of mast cells and inflammation. Another study found that the mast cell count in the tuberculoid leprosy group was significantly lower than that in the LL group.^[8] LL type had mast cells with the lowest density compared to the BT and BB type. High density mast cells in BT and BB type refer to the role of mast cells in the activation of immune response to *M. leprae* infection.^[9,10] Further study showed that Hsp70 which induced mast cell activity was detected in skin and nerve lesions from all leprosy patients but particularly prominent in lesions from patients undergoing RRs.^[11]

Mast cells have been known to induce variety of cytokines, such as TNF- α , IL-1, IL-4, IL-5, IL-6, and GM-CSF. Bagwan et al. found that mast cells in LL type were denser than TT type. It was associated with Th2 predominant as a cytokine response in LL type, and Th1 predominant in TT type.^[12] Moreover, in BL type, both Th1 and Th2 were produced consistent with mast cell density in the lesion; so that could explain the high incidence of RR in this type. It was found that there were many spindled and elongation of mast cells in BT type which indicated that mast cells were ready to degranulate.^[10]

In this study, there were some limitations such as research design and method of identifying mast cell levels that could affect the study results. The authors suggest future prospective study and other methods such as polymerase chain reaction (PCR) for identifying mast cell levels and role in leprosy patient with RR. There are also a lot of variables associated with RR that could not be assessed in this study by the cause of lack of funding. In addition, insufficient literature about variables studied in leprosy patients lead to the difficulty in determining the normal values. This study is not a discussion about the prevention of mast cell degranulation which will prevent RR on leprosy, anti-inflammation drugs on RR, mast cells role on RR in patients who have completed MDT. That may be the consideration of future studies.

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