ORIGINAL ARTICLE

Difference of Tumor Necrosis Factor (TNF-α) Levels in Multibacillary Leprosy between Reversal Reaction and Non-reversal Reaction Patients

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ABSTRACT

Background: Reversal reaction (RR) is an acute on chronic episode which frequently contribute to withdrawal of treatment and disability in leprosy patients. Reversal reaction has been shown associated with increase in TNF-α levels. TNF-α is a cytokine taking part in neutrophil and monocyte recruitment, also activate them to eliminate microbial infection.

Aim: This study is aimed at understanding the difference of TNF-α levels in both multibacillary leprosy patients with and without reversal reaction.

Methods: This cross-sectional study population was divided into two groups: leprosy patients with reversal reaction group (n = 28) and leprosy patients without reversal reaction (n = 28). TNF-α levels were measured by immunohistochemistry staining. The TNF-α levels in both groups respectively were analyzed using independent samples T test.

Result: This study showed a significant increase ($p<0.05$) in the expression of TNF-α in reversal reaction group compared with the non reversal reaction group.

Conclusion: This research showed that there was a significant difference of TNF-α levels produced by mast cells on skin lesion in patients with and without RR. This is consistent with the hypothesis of TNF-α in the RR patients.

Keywords: multibacillary leprosy, reversal reaction, TNF-α

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 mucous, upper respiratory airway, reticuloendothelial system, eyes, muscles, bone, testicles, and all of human organs other than central nervous system. Leprosy reactions are acute exacerbation manifest as activation constitutional symptoms and/or new skin efflorescence. There are 2 types of leprosy reactions: type 1 (Reversal Reaction / RR) and type 2 (Erythema Nodosum Leprosum / ENL).[1]

Reversal reaction is type IV hypersensitivity reaction which is frequently occurred to borderline forms leprosy as a result of cellular immune response to *M. leprae* antigen and characterized as an acute inflammation of former skin lesion. Approximately 95% of RR occurs simultaneously with diagnosis confirmation or during multi drug treatment (MDT). RR commonly appears on the first six months treatment, particularly in BT and BB leprosy. It also can be found in BL leprosy with longer interval during MDT. Clinical manifestation of RR includes abrupt increased in number and more active lesion with/ or 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].

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TNF-α is a cytokine taking part in neutrophil and monocyte recruitment, also activate them to eliminate microbial infection. Furthermore, TNF-α enhances endothelial cells adhesion to leukocytes.[3] Studies have determined that TNF-α mRNA and proteins levels produced by macrophages in BT and BL leprosy skin lesions are higher in RR patients than in non-RR patients.[4] In addition, TNF-α has been found to be higher in type 1 leprosy reaction compared to normal population and declined after reaction treatment.[5] Therefore, TNF-α measurement may become a useful predictive and monitoring parameter for reversal reaction. However, there were no studies which compared TNF-α produced by mast cell in RR and non-RR leprosy patients.

MATERIALS AND METHODS

Research design in this study was analytic observational with cross sectional study. Subjects were multibacillary leprosy patients being treated at the Donoharjo Hospital, Jepara. Selection of subject has done by consecutive sampling with double blind treatment. Participants were multibacillary leprosy patients who met WHO criteria and aged between 20-60 years old. Participants were divided into two groups; leprosy patients with reversal reaction group and leprosy patients without reversal reaction. Informed written consent was obtained from all individuals included in the study. Exclusion criteria included pregnancy, and other acute inflammatory diseases. Based on the sample calculations, total subjects in this study were 56 samples: leprosy patients with reversal reaction group (n = 28) and leprosy patients without reversal reaction (n = 28). TNF-α levels were measured by immunohistochemistry staining. The TNF-α levels in both groups respectively were analyzed using independent samples T test.

The data were analyzed using normality and homogenity test for age and occupation. Confounding factors in this study were controlled by randomization proccess. The data were significance if p <0,05 with confidence interval 95%. This study was approved by Ethics Committee of Faculty of Medicine, University of Airlangga.

RESULTS

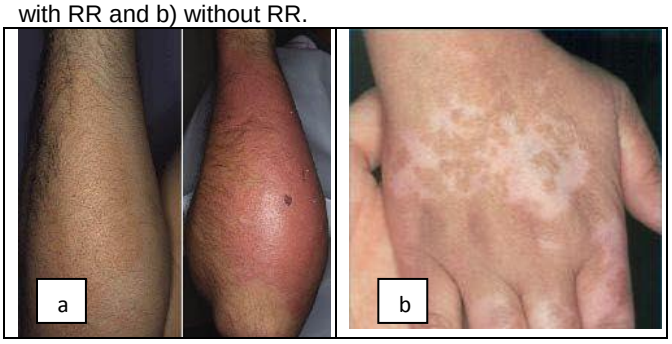
In this study, subjects age and sex were comparable in both groups.

Table 1. Subjects characteristics

	Non-Reversal Reaction	Reversal Reaction
Age		
N	28	28
Mean ± SD	41.00 ± 8.255	42.43 ± 9.126
Gender		
Men	15 (53.6%)	12 (42.9%)
Women	13 (46.4%)	16 (57.1%)

In this study we compared multibacillary leprosy with and without RR. Figure 1 was photograph that compares their clinical presentation.

Figure 1 Clinical presentation of multibacillary leprosy; a) with RR and b) without RR.



This study showed a significant increase (p <0.05) in the expression of TNF-α in reversal reaction group compared with the non reversal reaction group.

Figure 2: This picture shows mast cells that give a positive reaction to anti-TNF-α through immunohistochemical staining, x400, A: non reversal reaction group, B: reversal reaction group. Black arrow indicates the double stain to anti TNF-α. Red arrow indicates the mast cells that does not give a positive reaction.

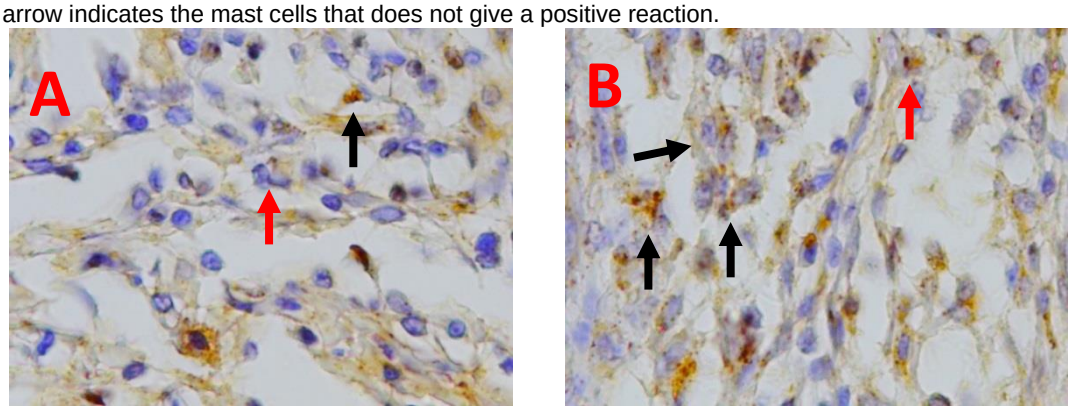


Figure 2: The histogram above shows increased expression of TNF-α in the mast cell of reversal reaction group compared with the non reversal reaction group

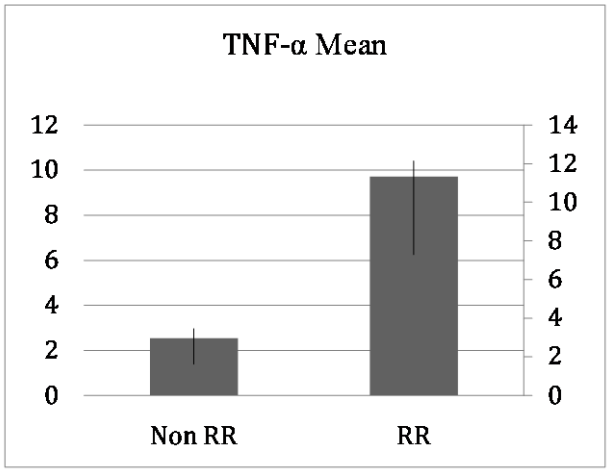


Table : Result of independent samples T test

Group	n	Mean ± SD	P value
Non Reversal Reaction	28	2.89 ± 1.257	< .001
Reversal Reaction	28	9.43 ± 2.911	

DISCUSSION

Reversal reaction is marked by delayed hipersensitivity to *M. leprae* antigen (Gell & Coombs type IV reaction) and abrupt increased in immune cell response. Theoretically, reversal reaction in terms of the germs destruction is a positive thing as it occurs massively. However, it is clinically dissatisfying because of acute inflammatory reaction which is aesthetically disturbing. Reversal reaction affects 20-30% of leprosy patients. RR occurs more frequent than ENL. Clinical manifestation of RR is acute inflammation of existing lesions, which can be erythematous, enlarged or transformed into infiltrat.[6]

In this study, TNF-α was examined by comparing variables between leprosy patients with RR and without

RR. There was a significant difference ($p < 0.001$) of TNF-α in both groups.

M. leprae infection may lead to the expression of MHC II on the surface of the cells. This may give rise to antigen presentation which triggers CD4 lymphocyte killing of the infected cell which is mediated by cytokines such as tumor necrosis factor (TNF).[7] Local tissue damage is one of the hallmarks of reversal reactions. This is probably caused in part by TNF-a, which has tissue-damaging effects such as stimulating the production of reactive oxygen intermediate metabolites, stimulating collagenase production, activating fibroblasts and promoting production of adhesion molecules[8].

IFN-γ is locally produced by CD4+ lymphocytes, respectively induces macrophages to produce TNF-α.[9] TNF-α may play a role as autocrine and auto-amplification in supporting the macrophage accumulation and differentiation. This positive feedback increases local tissue damage that is consistent with clinical experience of reversal reaction. The damage can be developed with only small amounts of antigen but require high doses of steroids to decrease the effects[10]

Previous studies have proved that the amount of TNF-α mRNA and proteins that were produced by macrophages in BT and BL leprosy skin lesions are higher in RR patients than in non-RR patients.[4] The study comparing the difference between TNF-α produced by mast cells in patients with leprosy reversal reactions and non reversal reaction is not yet known.

Previous study showed that RR was predominated by Th-1 cytokines such as TNF, IL-2, IFN-γ.[11] Macrophages would produce cytokines such as TNF-α, NO, and other substances under IFN-γ/STAT1 pathway.[12] Anti-inflammatory cytokines such as IL-10 and IL-13 were also observed in RR skin lesion.[13] IL-10 was produced by regulator macrophage and regulatory T cell.[14]

Mast cells are an important source in the production of TNF-α. Previous studies examined the relationship between mast cells and leprosy bacilli in the foot skin of immunosuppressed mice inoculated by leprosy bacilli.[8] The study showed that there were changes in the structure and morphology of mast cells in which structural changes 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 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 as plasma proteins such as albumin to form a histamine release peptide that will further trigger the activation of mast cells and inflammation.[15] Another study found that 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 refers to the role of mast cells in the activation of immune response to *M. leprae* infection[16].

This research showed that there was a significant difference of TNF-α levels produced by mast cells on skin lesion in patients with and without RR. This is consistent with the hypothesis of TNF-α in the RR patients. In this study, some limitations such as research design could affect the study results. There are also a lot of variables associated with reversal reaction such as Hsp-70, TLR-4, IL-6, and IL-8 that could not be assessed in this study by reason of lack of funding. In addition, insufficient literature about variables studied in leprosy patients leads to the difficulty in determining the normal values of the variables.

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