

Difference of Histamine Expression between Multibacillary Leprosy with and without Erythema Nodosum Leprosum (ENL) Reaction

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Abstract Leprosy is a chronic infectious disease that poses a problem for public health because it causes disability. One of the causes for this disability is leprosy reaction. Erythema Nodosum Leprosum (ENL) is a type 2 leprosy reactions, which were more frequent in borderline lepromatous (BL), and lepromatous (LL) type of leprosy receiving multi drug therapy (MDT) for multibacillary leprosy (MB). Histamine, primarily H4 receptor-mediated, plays role in inflammation by increasing vascular permeability and muscle contraction resulting in leukocytes migration and leakage of plasma containing antibodies and tissue complements. This cascade finally results in tissue inflammation. To our knowledge, there is no previous study analysing histamine expression difference between MB leprosy with and without ENL reaction. The purpose of the research was to understand the difference of histamine expression in MB leprosy patients with and without ENL reactions who received MDT for at least 6 months. The method of the research was an observational study with cross-sectional approach. Study samples were 32 people divided into 2 groups, 16 samples in without ENL reactions group and 16 samples in with ENL reactions group. Histamine expression was measured based on Allred scoring system. The result from Difference test for histamine expression between two study groups using Mann Whitney Test showed $p < 0.05$ ($p=0.030$). The study found that mean histamine expression of ENL MB patients was higher than non-ENL MB patients.

1 INTRODUCTION

Leprosy is a chronic infectious disease caused by *Mycobacterium leprae* (*M. leprae*) that were initially attacked peripheral nerves, then attacked the skin and other tissues, but excepted the central nervous system (Kosasih, A., Wisnu., I.M., and Daili, E.S., 2011 and Lee, D.J., Rea, T.H., Modlin, R.L., 2012). Leprosy reaction is an acute episode in chronic course of leprosy. It is an immune reaction, an antigen-antibody reaction. Since they often result in disfigurements, leprosy reactions have to be treated carefully. Leprosy reactions consisted of two different types, specifically type 1 or reversal reaction (RR) and type 2 or erythema nodosum leprosum (ENL) (Odom, R.B., James, W.D., and Berger, T.G., 2010; Lockwood, D.N.J., 2010; and Silva, M.R. and Castro, M.C.R.D, 2012).

Coomb and Gell suggested that type 2 reaction is a type III hypersensitivity reaction. The remains of

deceased bacilli after multi-drug therapy (MDT) result in antigen (Odom, R.B., James, W.D., and Berger, T.G., 2010; Lockwood, D.N.J., 2010; nd Daili, E.S., 2011 and Lee, D.J., Rea, T.H., Modlin, R.L., 2012). The antigen involves in reactions with IgM, IgG, and C3 complement, forming immune complexes that spread through blood circulation and attach in the end organs. This cascade results in complement system activation, producing anaphylatoxin and promoting degranulation of mast cell to release histamine. Histamine release will cause inflammation, primarily after activation of histamine h4 receptor (Frenzel, I. and Hermine, O., 2013).

After phagocytosis, the macrophage-eaten *M leprae* can produce Hsp70 during the stressful phagolysosom creation. Antileprosy drugs that given during the course of the therapy, add more pressure to bacilli inside the macrophages. Hsp70 accumulation inside the macrophages invites the cytotoxic lymphocytes to lyse the macrophages and activates

Table 1: The difference of mean histamine expression between leprosy patient with and without ENL reaction.

	Non ENL Reaction		ENL Reaction		P
	Mean±SD	Median (Min-maks)	Mean±SD	Median (Min-maks)	
Histamine expression	6,31 ± 1,35	6(4 – 8)	7,31±0,48	7 (7-8)	0,030 ^b

Note: b. *Mann-Whitney Test*

pro-inflammatory cytokines, such as TNF α . After the lysis, released Hsp70 attach to the TLR4 receptors on the surface of mast cells. The next process is activation of cell mast by the TLR4, as indicated by the histamine formation, TNF α , IL-6, and IL-8 then finally promote the inflammation (Mortaz, E., *et al*, 2007).

Immunohistochemistry is a method to detect the existence of specific antigen in a tissue based on antibody (Ab) and antigen (Ag) binding. ENL is an inflammatory reaction in tissue. Histamine-bound histamine h4 receptors will be captured by histamine h4 receptor antibodies and create histamine expression on immunohistochemistry technique (Trufelli, D.C., *et al*, 2010; Lin, Fan and Shi, Jianhui, 2015; and Park, C.S., Roh, J., and Kim, S.W., 2016).

Previous study have address the role of mast cell in leprosy reaction have been conducted. They found higher density of mast cell in leprosy reactions (Antunes, S.L.G., *et al*, 2003). Therefore, the objective of the study was to evaluate the histamine expression difference between MB leprosy with and without ENL reaction.

2 METHODS

This cross-sectional observational study was conducted in outpatient setting of leprosy clinic at Donorojo Leprosy Hospital, Jepara. The subjects were patients with multibacillary (MB) infection and aged 20-65 years old. To be included, the patients must have been received MDT for more than 6 months. During study period (March 2017- June 2017), 32 patients were included, 16 MB patients without ENL reaction and 16 MB patients with ENL reaction. The skin tissue samples were processed and analysed by immunohistochemistry method in anatomical pathology laboratory of Medical Faculty, Gadjah Mada University, Yogyakarta. Immunohistochemical examination and measurement of histamine expression can be seen using a light

microscope at 10 fields of view using 400x magnification. Each field of view will be photographed and then analyzed using a computer (image raster software) to calculate the percentage and intensity of cells that bind histamine h4 receptor antibodies, which then according to the percentage and the intensity of the staining will be incorporated into the semi-quantitative system by using the Allred scoring system

3 RESULT

In non-ENL group, the number of female and male patients was equal, the mean age was 42.19 ± 2.22 years, mean leukocyte count was 7806.25 ± 2094.54 /mm³, mean MDT duration was 8.5 ± 2.22 months. In ENL group, all of the patients were males, the mean age was 32.63 ± 11.93 years, mean leukocyte count was 8587.5 ± 1872.92 /mm³, mean MDT duration was 8.96 ± 2.024 months. The mean histamine expression in non-ENL and ENL group were 6.31 ± 1.35 and 7.31 ± 0.48 , respectively.

The present study found that mean histamine expression of ENL MB patients was higher than non-ENL MB patients. The Mann-Whitney test for mean histamine expression found significant difference ($p < 0.05$) with $p = 0.030$.

4 DISCUSSION

In this study we obtained that male leprosy patients were more than female where in multibacillary leprosy group without ENL reactions the number of men was eight persons (50%) and 16 persons (20%) in multibacillary leprosy group with ENL reaction. Gender generally is not a risk factor of ENL.

Sample distribution by age was found to be statistically significant. Erythema Nodosum Leprosum based on age group does not represent a particular group risk for developing ENL.

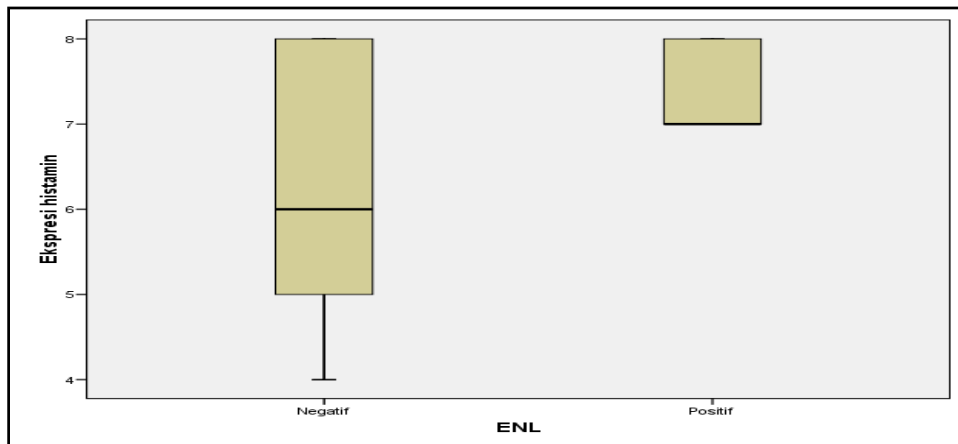


Figure 1: The study found that mean histamine expression of ENL MB patients was higher than non-ENL MB patients.

Table 2: Characteristic subjects of the study.

		Non Reaksi ENL			Reaksi ENL			P
		N(%)	Mean±SD	Median (Min-maks)	N(%)	Mean±SD	Median (Min-maks)	
Gender	male	8(50)			16(100)			0,002 ^a
	female	8(50)			0(0)			
Age			42,19±11,2	40(23-59)		32,63±11,93	31,5(20-59)	0,011 ^b
leukocytes			7806,25±2095,54	7750(3700-10700)		8587,5±1872,92	9100(4400-10900)	0,275 ^c
MDT Duration			8,5±2,22	8(6-12)		8,96±2,024	8(6-12)	0,688 ^b
Job	student	0(0)			2(12,5)			0,053 ^a
	non job	3(18,8)			7(43,8)			
	farmer	1(6,3)			0(0)			
	Labor	0(0)			1(6,3)			
	Retirement	0(0)			1(6,3)			
	driver	0(0)			1(6,3)			
	fisherman	1(6,3)			0(0)			
	Housewife	7(43,8)			0(0)			
	Private	4(25)			3(18,8)			
	employe	0(0)			1(6,3)			
Embankment farmer								

Note: a. Uji chi square ; b. Mann Whitney test ; c. Uji t independen

Leprosy is known to occur in all ages ranging from infancy to old age (3 weeks to over 70 years). But the most is happened at a young and productive ages. ENL occurs at any time during leprosy episodes and most often after 6 months treatment (Triamchaisri, S., *et al*, 2015).

Subjects of the study were limited by the number of leukocytes <11.000 / mmk to exclude inflammatory and infectious diseases in which histamine expression increased in the presence of inflammatory and infectious conditions (Zampeli, E. and Tiligada, E., 2009). The mean difference of leukocyte counts of multibacillary leprosy study

subjects with ENL and without ENL was found to be insignificant (p = 0.275) showed that the leukocyte rates of sample was homogeneous.

MDT duration therapy of multibacillary leprosy group with ENL and without ENL was found to be insignificant (p = 0,688). Erythema Nodosum Leprosum can occur at any time during leprosy course but most often occurs after 6 months of MDT treatment, since many leprosy bacteria are destroyed, that means many antigens are released and react with antibodies, thus activates the complement system and results in ENL (Odom, R.B., James, W.D., and Berger, T.G., 2010; Lockwood, D.N.J., 2010; Lee,

D.J., Rea, T.H., Modlin, R.L., 2012; and Silva, M.R. and Castro, M.C.R.D, 2012).

Histamine expression value in MB group with ENL reaction was higher than MB group without reaction with significant differences ($p = 0.030$) by *Mann-Whitney* test. Histamine plays a role in immunoinflammatory response particularly in those with H4 receptor (mast cells, eosinophils, T cells and dendritic cells) that activates immune cell maturation, increases cytokine production and increases cellular chemotactic response (Gutzmer, R., *et al*, 2011 and Harvima, I.T. and Nilsson, G., 2011). Histamine increases in inflammatory states such as ENL. Histamine expression in MB leprosy group with ENL reaction was higher than MB leprosy group without ENL reaction.

5 CONCLUSION

There was a significant difference in histamine expression between MB leprosy with and without ENL reaction where the mean of histamine expression in MB leprosy group with ENL reaction was higher than leprosy group without ENL reaction.

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