The Correlation between Neutrophil CD64, Interleukin-17 (Il-17), Interleukin-10 (Il-10) in Skin Tissue and Neutrophil CD64, Il-17 and Il-10 in Blood Circulation in Erythema Nodosum Leprosum (ENL) Patients

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Abstract: Erythema nodosum leprosum (ENL) remain major health problem globally. Current pathogenesis of ENL not only involving immune complex, but also neutrophil and cellular immune response. Neutrophil CD64, interleukin-17 (IL-17) and interleukin-10 (IL-10) are mediators that suggested to be involved in regulation of ENL. This study aimed to determine the correlation between neutrophil CD64, IL-17 and IL-10 in skin tissue with neutrophil CD64, IL-17 and IL-10 in blood circulation. This was an analytical observational study with a cross-sectional design which involved 30 patients of multibacillary (MB) type of leprosy patients which underwent ENL. Neutrophil CD64 levels in blood circulation were measured by flow cytometry, while IL-10 and IL-17 levels in the blood circulation with ELISA. Biopsy was performed prior to the examination of neutrophil CD64, IL-17 and IL-10 from skin tissue with immunohistochemistry (IHC). The obtained data were analyzed descriptively and with correlation analysis. The mean of neutrophil CD64, IL-17 and IL-10 in skin tissue was 19.10 ± 5.371, 11.47 ± 5.029 and 6.73 ± 3.039, respectively, while the mean of neutrophil CD 64, IL-17 and IL-10 in circulation was 66.807 ± 14.794, 59.55 ± 36.397 and 186.30 ± 272.974, respectively. No significant correlation was found between IL-17, neutrophil CD64 and IL-10 in skin tissue with IL-17, neutrophil CD64 and IL-10 in blood circulation with significance level of 0.511, 0.186 and 0.401, respectively. There was no correlation between dysregulation of local immunity with systemic immunity in ENL.

1 INTRODUCTION

Erythema nodosum leprosum (ENL) is one type of leprosy reactions. Current world health organization (WHO) multidrug therapy (MDT) had not been able to eliminate the incidence of leprosy reactions yet. The prevalence of ENL cases varies greatly depends on geographical variations. ENL cases in Asia were high which varied from 19% to 26% among all borderline lepromatous (BL) and lepromatous leprosy (LL) patients (Kahawita et al., 2008).

To date, pathogenesis of ENL not only involving immune complex. Neutrophil and cellular immune response were found to be greatly involved in the pathogenesis of ENL. Neutrophil is the main inflammatory cells infiltrate in ENL. The accumulation of neutrophil in skin tissue caused by the increase of E-selectin expression in the endothelium due to interleukin-17 (IL-17) and interferon γ (IFN-γ) stimulation. A study by Schmitz found that the active neutrophil cells were characterized by increased expression of Fc gamma receptor I (FcγRI) or cluster of differentiation 64 (CD64). CD64-expressing neutrophils were found to be increased either in blood circulation or skin tissue

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of ENL patients and related to the severity of ENL (Schmitz et al., 2016).

Another molecular factor which found in ENL and involved in nerve damage regulation due to leprosy is interleukin-10 (IL-10). Lipid droplet (LD), found in lepromatous type of leprosy patients, is an active catalyst place for the synthesis of prostaglandin-E2 (PGE-2) which promote the production of IL-10. Either PGE-2 or IL-10 involved in suppressing the inflammation in the nerve. LD in Schwann cells (SC) indirectly acts as protective factor to maintain SC from damage caused by nerve inflammation (Mattos et al., 2011; Mattos et al., 2010).

Previous study reported that neutrophil CD64 increased in blood circulation or skin tissue of ENL patients and related to the severity of ENL (Schmitz et al., 2016). Either IL-17 or IL-10 also involved in the pathogenesis of ENL. Nerve damage, which occurred in ENL, is assumed to be affected by the dysregulation of systemic and local immunity. Thus, this study aimed to analyze the correlation between local mediators (neutrophil CD64, IL-17 and IL-10 in skin tissue) with systemic mediators (neutrophil CD64, IL-17 and IL-10 in circulation) in ENL.

2 METHODS

This was an analytical observational study with cross-sectional design conducted at Dr. Soetomo General Hospital, Surabaya, Indonesia, during July to September 2017. This study involved 30 patients with multibacillary (MB) type of leprosy who suffered ENL leprosy reaction, based on calculation with sample size formula. Consecutive sampling was used to obtain the samples. The inclusion criteria were patients with MB type of leprosy with ENL which defined clinically and bacteriologically, aged between 18 to 60 years and willing to participate in this research by signing the informed consent. Whereas patients with a pregnant condition or poor general condition, history of peripheral nerve lesion/trauma, and having a history of oral thalidomide treatment in the last two days were excluded.

2.1 Measurement of Neutrophil CD64, IL-10 and IL-17 in Circulation and IL-17, Neutrophil CD64 and IL-10 in Skin Tissue

The blood sample was taken for examination of neutrophil CD64, IL-10 and IL-17 levels in circulation. Neutrophil CD64 measured by flow cytometry while ELISA was used for IL-10 and IL-17 measurement.

The sample of skin tissue was taken from foot region with punch biopsy. The procedure of the biopsy described as follows; disinfection was performed with 70% of alcohol on the foot region of the patients. Subsequently, local anesthesia with 0.25 ml of lidocaine was done. On that area, biopsy with punch method was performed with diameter of 4 mm. The obtained skin tissue was put in the eppendorf containing 10% formalin. The wounded area cleaned with 0.9%NaCl, applied with a topical antibiotic and covered with sterile gauze. The samples then processed and immunohistochemistry (IHC) method was used to examine IL-17, neutrophil CD64 and IL-10 expressions (by counting on 20 fields of view in 1000 times magnification by microscope, with results of ratio-scale data). Measurement of IL-10 in skin tissue in this study was specified to IL-10 expressed by Schwann cells (SC), through staining of S-100 protein as a marker of SC.

2.2 Data Analysis

Data were analyzed with Statistical Package for the Social Sciences (SPSS) for Windows version 22.0. The univariate/descriptive analysis was conducted to describe the subject characteristics and research variables. The numerical data presented in mean and standard deviation (SD), while the categorical data with relative frequency or amount and percentage. The result of the descriptive analysis presented in single distribution table. Spearman or Pearson correlation analysis was performed to determine the correlation between variables in blood circulation and skin tissue. A *p*-value of less than 0.05 was considered significant.

3 RESULT

3.1 Characteristics of Study Subjects

Characteristic of study participants are age, sex, occupation, type of leprosy, type of ENL reaction and the severity of ENL. Variables in skin tissue (neutrophil CD64, IL-17 and IL-10) and blood circulation (IL-17, neutrophil CD64, IL-10) are presented in Table 1.

3.2 Immunohistochemistry (IHC) Results

The results of IHC examination from skin tissue are depicted in figure 1.

Table 1. Characteristics of ENL patients at Dr. Soetomo General Hospital, Surabaya.

Number.	Variables	Respondents (N = 30)
1.	Age, n (%)	
	11-20 years	3 (10.0%)
	21-30 years	8 (26.7%)
	31-40 years	9 (30.0%)
	41-50 years	7 (23.3%)
	51-60 years	3 (10.0%)
2.	Sex, n (%)	
	Male	6 (20.0%)
	Female	24 (80.0%)
3.	Occupation	
	Housewife	3 (10.0%)
	Students/college students	3 (10.0%)
	Government employees	2 (6.7%)
	Private sector	22 (73.3%)
3.	Type of Leprosy, n (%)	
	LL	18 (60.0%)
	BL	12 (40.0%)
4.	Type of ENL reaction, n (%)	
	Acute	0 (0.0%)
	Chronic	30 (100.0%)
5.	The Severity of ENL, n (%)	
	Mild	9 (30.0%)
	Moderate	18 (60.0%)
	Severe	3 (10.0%)
6.	Variables in skin tissue, Mean ± SD (CI)*	
	Neutrophil CD64	19.10 ± 5.371 (17.09–21.11)
	IL-17	11.47 ± 5.029 (9.59–13.34)
	IL-10	6.73 ± 3.039 (5.60–7.87)
7.	Variables in circulation, Mean ± SD (CI)**	
	Neutrophil CD64	66.807 ± 14.794 (61.283–72.331)
	IL-17	59.55 ± 36.397 (45.962–73.144)
	IL-10	186.30 ± 272.974 (84.373–288.233)

**Normal distribution of data with Kolmogorov Smirnov test of 1 sample; **Normal distribution of neutrophil CD64 data but not for IL-17 and IL-10 with Kolmogorov Smirnov test of 1 sample. CI: Confidence interval.*

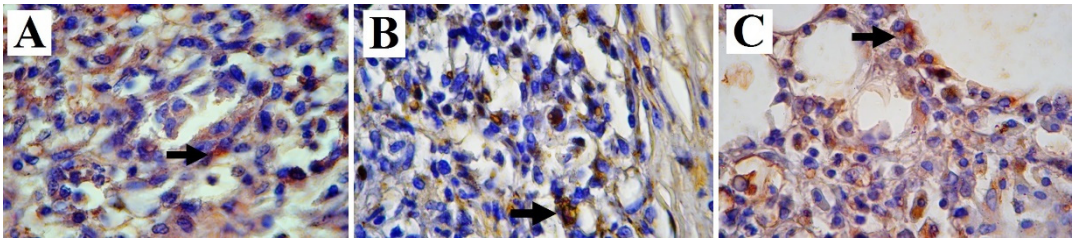

Figure 1: Skin tissue biopsy with IHC staining using: A. Anti-human IL-17 monoclonal antibody; B. Anti-human CD64 monoclonal antibody; C. Anti-human IL-10 and anti-S-100 monoclonal antibody (black arrows = positive reactions) (1000x magnification).

Table 2: The correlation between variables in skin tissue with variables in circulation.

Variables	r	Significance
IL-17 in skin tissue IL-17 in circulation	0.125	0.511*
Neutrophil CD64 in skin tissue Neutrophil CD64 in circulation	0.248	0.186**
IL-10 in skin tissue IL-10 in circulation	0.159	0.401*

*Spearman; **Pearson

3.3 The Correlation of IL-17, Neutrophil CD64 and IL-10 in Skin Tissue with IL-17, Neutrophil CD64 and IL-10 in Blood Circulation

Correlation analysis between variables in skin tissue (IL-17, neutrophil CD64 and IL-10) with variables in circulation (IL-17, neutrophil CD64 and IL-10) was performed. There is no significant correlation between IL-17, neutrophil CD64 and IL-10 in skin tissue with IL-17, neutrophil CD64 and IL-10 in blood circulation with significance level of 0.511, 0.186 and 0.401, respectively (table 2).

4 DISCUSSION

This study found that neutrophil CD64 in skin tissue did not correlate with neutrophil CD64 in blood circulation. Few CD64 was expressed on the surface of neutrophil (average of 1400 receptors per cell) in inactive neutrophil. The increased expression of CD64 on the surface of neutrophil as a marker of active neutrophil occurs in four until six hours after stimulation of IFN-γ or granulocyte-macrophage colony-stimulating factor (GM-CSF) and other kinetic factors, e.g., lipopolysaccharide (Nuutila, 2010; Allen et al., 2002). CD64 surface receptors were not only expressed on neutrophil cells surface but also on monocytes. In this study, CD64 expressed by neutrophil in blood circulation was distinguished from CD64 expressed by monocytes through flow cytometry examination with anti-CD163 to mark out monocytes, thus only CD64 expressed by neutrophil was calculated.

Neutrophil CD64 in circulation entered skin tissue because of increased expression of E-selectin on endothelium. However, this study found no significant correlation between neutrophil CD64 in skin tissue with neutrophil CD64 in circulation with significance value of 0.186 (< 0.05).

IL-17 is an inflammatory cytokine in skin tissue which may promote the production of IL-6, IL-8 and GM-CSF from non-immune cells, e.g., fibroblast and epithelial cells via activation of nuclear factor-kappaB (NF-kB) transcription factor (Stettner et al., 2014). In general, IL-17 is involved in inducing pro-inflammatory chemokines, hematopoietic cytokines, acute phase response genes and anti-microbial substances. The current study found no significant correlation between IL-17 in skin tissue with IL-17 in blood circulation with significance value of 0.511 (> 0.05).

There is no study concerning the relationship between IL-17 in circulation with IL-17 in skin tissue of ENL patients. One study by Li et al. on Guillain-Barré syndrome patients reported a significant correlation was found between IL-17 in circulation with in cerebrospinal fluid. This may be due to IL-17 in circulation is able to penetrate the blood-brain barrier, hence its levels in cerebrospinal fluid also increased. The different result which obtained in this study may be due to IL-17 in circulation is unable to enter skin tissue in large quantities.

IL-10 is a cytokine with multiple and pleiotropic effect in the immunoregulatory process. This study found no significant correlation between IL-10 in skin tissue with IL-10 in circulation with a significance value of 0.401 (> 0.05). It has been suggested that this occur due to IL-10 in blood circulation is produced by monocytes and other cells, e.g., lymphocytes and mast cells. In this study, the levels of IL-10 in blood circulation measured by ELISA which detects IL-10 levels regardless of the cells that generate it, while the expression of IL-10 in skin tissue was specific to IL-10 which expressed by SC, through S-100 staining.

It has been assumed that aberration which occurs in ENL, e.g., nerve damage is related to dysregulation of systemic and local immunity, hence in this study the local mediators in skin tissue and systemic mediators in blood circulation were examined. This study obtained novel findings that there was no

correlation between dysregulation of local immunity with systemic immunity.

5 CONCLUSION

There was no correlation between dysregulation of local immunity with systemic immunity in ENL. Nerve damage in ENL is dominated by local processes. Further research is required to investigate local immune responses and to explore local intervention for management of neurological damage in ENL.

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