



23rd
RCD
Regional Conference of Dermatology
SURABAYA
2018

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CONFERENCE OF
DERMATOLOGY**
(Asian - Australasian)

Incorporating With
**The 16th Annual Scientific Meeting
of the Indonesian Society of
Dermatology and Venereology**

**Enhancing Evidence Based
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THE ROLE OF TRANSFORMING GROWTH FACTOR- β AND IMMUNOGLOBULIN-M ANTI PHENOLIC GLYCOLIPID-1 WITH RECURRENT ERYTHEMA NODOSUM LEPROSUM

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Abstract

Recurrent Erythema Nodosum Leprosum (ENL) is a serious complication of leprosy immunology, that cause the inflammation of the skin, nerves and other organs. The aims of this study examined the role of TGF- β and IgM anti PGL-1 with recurrent ENL on leprosy patients in the Dr. Abdul Moeloek Hospital Lampung and Dr. Rivai Abdullah Leprosy Hospital Palembang. By using a cross sectional comparative study, examined the subject serum level of TGF- β and IgM anti PGL-1 by ELISA. With the result was a significant difference between the serum levels of TGF- β and IgM anti PGL-1 with the incidence of recurrent ENL reaction and unrecurrent reaction. The increasing levels of TGF- β and IgM anti PGL-1 level on patients with leprosy MB type can be as a predictor of the recurrent ENL.

Keywords: ENL, TGF- β , IgM anti PGL-1.



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INTRODUCTION

Erythema Nodosum Leprosum (ENL) is a serious complication of leprosy immunology, that cause the inflammation of the skin, nerves and other organs. The cause and risk factor of ENL were the immunology complication reaction on leprosy. Some of those were caused by the deposition of the *M. Leprae* antigen and complex antibody (¹). ENL can cause deformity and disability that make the quality of life decreased. onset of ENL reaction mediated by antigen-antibody immunological reactions in accordance with the hypersensitivity reaction type III according Comb and Gell (^{2,3}). Good handling on ENL will reduce the number of disability. ENL may arise before and during treatment, even in the completion of treatment (^{2,4}).

At the time of ENL reaction there was increases in serum Transforming growth factor (TGF- β), interferon gamma (INF- γ), interleukin-10 (IL-10), IL-6, IL-8 and IL-1B. While IL-4 and IL-5 remained unchanged (⁵). TGF- β is a product of the macrophages that have been activated and the most interesting cytokine because it has a large immunoregulatory function and doubles acts (⁶). TGF- β can regulate a variety of immune cells such as lymphocytes, macrophages and dendritic cells. TGF- β has a strong immunosuppressive effect on B cells, T cells CD41, T cells CD81, APC and macrophages (⁷).

In leprosy patients who received Multi Drugs Therapy (MDT) treatment will lead the *M lepra* experiencing fragmented, one of the bacteria that are part of that Phenolic-glycolipid antigen-1 (PGL-1). The PGL-1 antigen will stimulate the formation of antibodies IgM anti PGL-1, this antibody reacts with newly formed PGL-1 antigen and this will cause an ENL reaction (⁸). Acute symptoms found in ENL are suspected to sign the increasing natural excessive immune function, reflected by an increase in TNF- α , TGF- β and antibody IgM anti PGL-1.

The presence of antibodies to PGL-1 related to the bacterial index in *M. leprae* (BI) patients, at the time of ENL reaction the levels of IgM antibody anti PGL-1 are also increased (). Examination of IgM anti PGL-1 may also be useful as a determinant of early diagnosis and prognosis of leprosy. (¹⁰).

The main treatment of ENL reaction is corticosteroids, where the majority of the provision in the long term can reach 2-3 months. Steroid works by inhibiting the inflammatory processes in early phase and late-phase and decreasing neutrophil chemotaxis and inhibiting prostaglandin synthesis. At the time of ENL reaction improved and cured, steroid administration dose can be reduced or discontinued. At the time of dose reduction, recurrent ENL reactions often occurred so that the steroid dose should be administered again in original dose (¹¹). This study is aimed to find out relationship TGF- β AND IgM antibody anti PGL-1 with recurrence of ENL.

MATERIALS AND METHOD

This study was a cross-sectional comparative study, to determine the relationship of serum levels of TGF- β with the incidence of recurrent ENL reaction based on IgM antibody anti PGL-1 and Cortisol. The subjects were leprosy patients at Dr.H. Abdul Moeloek hospital Lampung and dr. Rivai Abdullah Leprosy hospital Palembang, aged between 18-60 years old and clinically and laboratory determined based on WHO standards (¹⁴), have received treatment with corticosteroids for 2 months and are not suffered from lung tuberculosis and diabetes mellitus, and are not pregnant and breast-feeding for female patients. Both groups were examined their serum levels of TGF- β by ELISA using Human TGF- β Bio legend kit (USA) AND IgM antibody anti PGL-1 with



the Laboratory of Leprosy Institute of Tropical Disease kit , Airlangga University Surabaya, Indonesia .

The statistical analysis using t-independent, to know the differences in subject characteristics between groups with the recurrent ENL reaction and unreccurent ENL reaction, T-independent test was done to know the difference between TGF- β , IgM antibody anti PGL-1 in the subject of study with recurrent ENL reaction and unreccurent ENL reaction, and test of logistic binary regression to determine the relationship between TGF- β AND IgM antibody anti PGL-1 on the incidence of recurrent ENL reaction.

This study was approved by the Ethics Committee of the Faculty of Medicine,University of Lampung,Indonesia.

RESULT

The number of subjects were 44 people consisted of 22 MB types leprosy patients with recurrent ENL reaction and 22 patients of unreccurent ENL reaction, between ages of 18- 60 years old (mean 34.9 ± 13.6) there where more males than females with a ratio of 7 : 3 (table 1).

Table 1. The Characteristics of recurrent ENL reaction by Age and Gender

Variable	ENL reactions					
	Recurrent			Unrecurrent		
	Total	%	mean+SD	Total	%	mean+SD
Age group						
< 20	4	18.2	34.9 ± 13.6	1	4.6	47 ± 18.4
21-30	7	31.8		3	13.6	
31-40	5	22.7		7	31.8	
41- 50	2	9.1		6	27.3	
> 50	4	18.2		5	22.7	
Gender						
Male	16	72.7		15	68.2	
Female	6	27.3		7	31.8	

From this study we found that the levels of BI/MI in patients with recurrent ENL reaction obtained with the biggest level < 3+ for 16 patients (72,8%) and the level of ≥ 3 only 6 patients (27,2%), but in control most of them in level < 3+ for 21 patients (95,4%). And according to type of leprae we found BL type bigger than LL type (63,4%) (table 2)



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Table 2. Characteristic subject according to clinical finding

Characteristic subject	Cases		Control		p value
	F	%	f	%	
BI/MI					
< 3+/0	16	72,8	21	95,4	0,075
≥ 3+/0	6	27,2	1	4,6	
Type of Lepra					
BL	14	63,4	18	81,8	
LL	8	36,6	4	18,2	

The results of the mean level's serum of TGF-β by ELISA in patients with recurrent ENL reaction obtained at the levels of 62.6 ± 30.4 pg / ml with the highest concentration of 134.5 pg/ml and the lowest of 23.3 pg/ml, and patients with unrecurrent ENL reactions gained the mean at levels 47.2 ± 23 pg/ml.

The results of the examination mean serum levels of antibodies IgM anti PGL-1 by ELISA in patients with recurrent ENL reactions gained $2,029 \pm 1,687$ μ / ml with the highest levels of 5.702 μ/ml and the lowerest of 150 μ/ml and in patients with unrecurrent ENL reactions gained the mean levels 629 ± 1043 μ/ml. (table 3)

Table 3. The difference of profile TGF-β and IgM anti PGL-1 the subject

Variabel	Group	Mean	SD	Min	Max	p value
TGF-β (pg/ml)	Recurrent ENL	62.6	30.4	23.3	134.5	0.015
	Unrecurrent ENL	47.2	23	10.7	88	
IgM anti PGL-1 (μ/ml)	Recurrent ENL	2,029	1,687	150	5,702	0.001
	Unrecurrent ENL	629	1,043	125	5,241	

The statistical t-independent test of TGF-β, IgM anti PGL-1 and Cortisol of leprosy group's influence to recurrent ENL reaction and in unrecurrent ENL reaction had value of $p = 0.015$, 0.001 and 0.035 ($p < 0.05$). This means that statistically there was a significant difference between serum levels of TGF-β and IgM anti PGL-1 with the incidence of recurrent ENL reaction and unrecurrent reaction (table 3).

DISCUSSION

Age and gender

In a study conducted on 44 subjects consisting of 22 types of MB leprosy patients who get recurrent ENL reaction and 22 patients with type MB who do not get ENL reaction as control, doing treatment at Dr. H. Abdul Moeloek Hospital Lampung and Dr. Rivai Abdullah Leprosy Hospital Palembang, it was obtained 16 (72.8%) males and 6



(27.2%) females as sample, while in the control group gained 15 males and 7 females. This study is similar to the studies in India, where the distribution of the male gender in patients with ENL amounted to 74.2% (¹⁵).

It can be explained because the type of MB leprosy patients are the most vulnerable to ENL suffered by men, so that ENL cumulatively suffered by males with MB leprosy. In female with MB type leprosy, pregnancy and childbirth is the originator of ENL reactions (¹⁶).

Based on the age of the group with recurrent ENL reaction of most age was 21-30 years old which accounted for 7 (31.8%) sample, while in the control group most at the age of 31-40 years was accounted for 7 (31.8%) sample.

In the broader population of leprosy patients who most often attacked by ENL lies in the age range under 40 years, the prevalence in India for patients with ENL under 40 years is 84% (¹⁷), it should be placed in treating patients with leprosy type MB in the age group under 40 years with wary reactions of ENL and also noticed other predisposing factors, such as bacterial index and type of leprosy.

Bacterial Index

In this study, bacteria index < 3+ by 72% in patients with type MB leprosy who get recurrent reactions. Semiquantitative bacterial index is a measure of the presence of bacteria in the patient's body, and becomes a parameter in the ENL. Referring to ENL based on the theory of immune complexes, the only source of antigens derived from bacteria *M. leprae* in the patient's body, reflected by the index measuring the bacteria (¹⁸). But this study is different from the other study in which most bacteria index < 3+, it is probably because ENL happened on this study the subject has been recurrent and ever received treatment, or it is suspected that may be other mechanisms that influence the occurrence of recurrent ENL reaction.

Recurrent ENL relationship with TGF- β

At the time of ENL reaction there were also increases in serum Transforming growth factor (TGF- β), interferon gamma (INF- γ), interleukin-10 (IL-10), IL-6, IL-8 and IL-1B. While IL-4 and IL-5 remained unchanged (⁵).

In this study of 22 patients, who experienced recurrent ENL reaction, we obtained the highest levels of TGF- β at a level of 50-100 pg / ml at 10 people (45.5%), with a mean of 62.6 ± 30.4 pg / ml, in contrast to the control group, with unrecurrent reaction, with the highest levels of < 50 pg / ml, which is 12 people (54.6%) with a mean of 47 ± 21.6 pg / ml. It is clear that the higher the levels of TGF- β in a patient's body, the more vulnerable the patient to experience repeated reaction of ENL. This study was the same as that obtained by Goulart, where the level of TGF- β in leprosy patients with ENL reaction is higher than those without reaction. TGF- β primes macrophages to express inflammatory gene product in response to particulate stimuli macrophage is most likely to encounter phagocytosable bacilli, so enhancing the inflammatory response (⁶). According to Kahawita there are several pieces of evidence for increased T-cell activity in LL patients with ENL in comparison with patients LL and without ENL (¹⁹), Gorelick in his study concluded that TGF- β mediates the inhibition of T cell differentiation into type 1 Th cell (⁷).

Statistical calculation of p value = 0.015 (p < 0.05), it means there is a significant difference of TGF- β levels in patients with leprosy incidence of recurrent ENL reaction to the unrecurrent reaction.



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Recurrent ENL relationship with IgM anti PGL-1

The genesis of ENL reaction is mediated by antigen-antibody immunological reactions in accordance with the hypersensitivity reaction type III according to Comb and Gell^(2,3). At the time of ENL reaction, there was increasing of antigen derived from a large number of dead bacteria *M. leprae* and reacted with antibodies in the body, there was also a decrease in the function of T suppressor cells⁽¹⁶⁾, one part of the bacteria is Phenolic-glycolipid antigen-1 (PGL-1). With the present of PGL-1 antigen it will stimulate the formation of IgM anti PGL-1 antibodies, this antibody reacts with newly formed PGL-1 antigen and will cause a ENL reaction⁽⁸⁾.

In this study of 22 patients who experience a recurrent ENL reaction, we obtained serum levels of antibody IgM anti PGL-1 with mean $2029 \pm 1687 \mu\text{ml}$, with the highest levels of $5.702 \mu\text{ml}$ and lowerest levels of $1.687 \mu\text{ml}$, but in control group that was unrecurrent reaction with a mean of $629 \pm 1043 \mu\text{ml}$. It is clear that the higher the levels of IgM anti PGL-1 in the body of the leprosy, the more vulnerable the patient to experience recurrent ENL reaction in this study, it was found a great standard deviation levels of antibody IgM anti PGL-1, due to the wide variety of data, it shows the heterogeneity of data.

Other studies such as Rojas found that the IgM anti PGL-1 antibody increased in leprosy with ENL reaction compared with unreacted ENL⁽⁸⁾, as well as Moura (2008) research which conclude IgM anti PGL-1 antibody can be a predictor of reaction⁽¹⁰⁾. While Silva et al (2007) research, getting no differences in levels of IgM anti PGL-1 in leprosy patients with ENL reaction or no reaction⁽²⁰⁾. Research Zenha et al (2003) found that levels of IgM antibodies anti PGL-1 in patients with leprosy is higher in patients who have not received treatment than those already received treatment⁽²¹⁾.

Statistical calculation of p value = 0.001 ($p < 0.05$), it means there is a significant difference levels of IgM antibodies anti PGL-1 in leprosy patients with recurrent ENL reaction compared with unrecurrent reaction.

CONCLUSION.

The conclusion of this research there was a significant differences between the levels of TGF- β , IgM anti PGL-1 and Cortisol on the incidence of leprosy patients with the recurrent ENL reaction to the unrecurrent ENL reaction.

There was a correlation between the levels of TGF- β with recurrent ENL based on the antibody IgM anti PGL-1. The increasing levels of TGF- β and IgM anti PGL-1 level in patients with leprosy type MB can be a predictor of reaction. .

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THE ROLE OF TRANSFORMING GROWTH FACTOR-B AND IMMUNOGLOBULIN-M ANTI PHENOLIC GLYCOLIPID-1 WITH THE RECURRENCE OF ERYTHEMA NODOSUM LEPROSUM

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Background

Recurrent ENL is a serious complication of leprosy immunology that can cause inflammation of the skin, nerves and other organs. The cause and risk factor of ENL were the immunology complication reaction on leprosy. Some of those were caused by the deposition of the M.Leprae antigen and complex antibody. TGF- β and IgM anti PGL-1 usually increase during recurrent ENL reaction.

Subjects

This study was 22 cases leprosy patients with recurrent ENL reactions and 22 cases unrecurrent reaction as controls.

Results

This study was from 44 subjects of leprosy MB type, there was 22 subjects with recurrent ENL reaction (16 male, 6 female, mean age 34.9) mean of TGF- β was 62.6 ± 30.4 pg/ml, IgM anti PGL-1 was 2029 ± 1687 μ /ml, and 22 subjects unrecurrent reaction as controls (15 male, 7 female, mean age 47) t mean of TGF- β level was 47.2 ± 23 pg/ml, and IgM anti PGL-1 was 629 ± 1043 μ /ml. The t-independent statistic test the influence of leprosy MB type group to the recurrent ENL reaction and the un-recurrent ENL reaction leprosy MB type patients to TGF- β and IgM anti PGL-1 $p < 0.05$ respectively.

Discussion

In this study of 22 patients who experience a recurrent ENL reaction, we obtained serum levels of TGF- β with mean 62.6 ± 30.4 pg/ml and antibody IgM anti PGL-1 with mean 2029 ± 1687 μ /ml (table 2). This means that there was a significant difference between the serum levels of TGF- β and IgM anti PGL-1 with the incidence of recurrent ENL reaction and unrecurrent reaction.

Conclusion

There was a significant differences between the levels of TGF- β , IgM anti PGL-1 on the incidence of leprosy patients with the recurrent ENL reaction to the unrecurrent ENL reaction. The increasing levels of TGF- β and IgM anti PGL-1 level on patients with leprosy MB type can be as a predictor of the recurrent

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Objective

This study was to examine the role of TGF- β and IgM anti PGL-1, with the incidence of recurrent ENL in leprosy patients in the Dr. Abdul Moeloek Hospital Lampung and Dr. Rival Abdullah Leprosy Hospital Palembang.

Methods

This study design was a cross sectional comparative study. This research examined the subject serum level of TGF- β and IgM anti PGL-1 by ELISA.

Table 1. The Characteristics of recurrent ENL reaction by Age and Gender the subject

Variable	ENL reactions					
	Recurrent			unrecurrent		
	Total	%	mean \pm S	Total	%	mean \pm S
Age group						
<20	4	18.2	34.9 \pm 13.	1	4.6	47 \pm 18.4
21-30	7	31.8		3	13.6	
31-40	5	22.7		7	31.8	
41-50	2	9.1		6	27.3	
>50	4	18.2		5	22.7	
Gender						
Male	16	72.7		15	68.2	
Female	6	27.3		7	31.8	

Table 2. The difference of profile TGF- β and IgM anti PGL-1

Variable	Group	Mean	SD	Min	Max	p value
TGF- β (pg/ml)	Recurren	62.6	30.4	23.3	134.5	0.015
	Unrecurre	47	23	10.7	88	
IgM anti PGL-1 (μ /ml)	Recurren	2.029	1.687	150	5.702	0.001
	Unrecurre	629	1.043	125	5.241	

