

Original Research Article

## The Relationship between Transforming Growth Factor-B and Immunoglobulin-M Anti Phenolic Glycolipid-1 with ENL Recurring Events

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### ABSTRACT

**Background :** Recurrent ENL is a serious complication of leprosy immunology that can cause inflammation of the skin, nerves and other organs. ENL can cause total disability and disability so that the quality of life is declined. TGF- $\beta$  and IgM anti PGL-1 antibody usually increase during recurrent ENL reaction.

**Aim :** This study was to examine whether there was a relationship between TGF- $\beta$  and IgM anti PGL-1 antibody with the incidence of recurrent ENL reactions in leprosy patients in the Dr. Abdul Moeloek Hospital Lampung and Dr. Rivai Abdullah Leprosy Hospital Palembang.

**Subjects :** This study was 22 cases (16 male, 6 female, mean age 34, 9) leprosy patients with recurrent ENL reactions and 22 cases (15 male, 7 female, mean age 47) unrecurrent reaction as controls.

**Methods :** For cases and control were examined their serum levels of TGF- $\beta$  by ELISA using Human TGF- $\beta$  Bio legend kit (USA) and IgM anti PGL-1 antibody with the Laboratory of Leprosy Institute of Tropical Disease kit, Airlangga University Surabaya Indonesia.

**Results:** The Statistical t-independent test of TGF- $\beta$ , the leprosy group's influence to recurrent ENL reaction and in unrecurrent ENL reaction with value of  $p = 0.015$  ( $p < 0.05$ ). The statistical t-independent test of IgM anti PGL-1, the leprosy group's influence to recurrent ENL reaction and leprosy unrecurrent reaction with  $p$  value = 0.000, ( $p < 0.05$ ). This means that there was a significant difference between serum levels of TGF- $\beta$  and IgM anti PGL-1 antibody with the incidence of recurrent ENL reaction and unrecurrent reaction.

**Conclusion :** In this study, the levels of TGF- $\beta$  and the levels of IgM anti PGL-1 antibody have significant difference between the incidence of leprosy patients with recurrent ENL reaction and unrecurrent reaction. Increased levels of TGF- $\beta$  and the levels of IgM anti PGL-1 antibody in patients with leprosy type MB can be a predictor of reaction.

**Keywords:** ENL, TGF- $\beta$ , IgM anti PGL-1.

### INTRODUCTION

Leprosy is a chronic infectious disease caused by slowly growth bacteria the *Mycobacterium leprae* (*M.leprae*) which

is an obligate intracellular invading Schwann cells and macrophages of the skin and peripheral nerves and other organs such as kidneys, eyes, testes, bone, muscle

(Fitness, 2002). This disease can cause permanent deformity and disability which in turns, it can cause social and economic problems.

Indonesia is still a country with the third highest incidence of leprosy patients in the world after India and Brazil. In 2012 the number of new cases recorded 18.994 people and the number of registered cases of 22.390 people and the prevalence rate of 0.86 per 10.000 population, and 80,96% of them were the multi bacillary (MB) type (WHO, 2013).

Erythema Nodosum Leprosum (ENL) is a serious complication of leprosy that can cause inflammation of the skin, nerves and other organs. Etiology and risk factors of ENL are complications of immune reactions in leprosy. This is partly caused by the deposition of *M.leprae* antigen and antibody complex (Fitness, 2002). ENL can cause total disability and disability so that the quality of life is declined onset of ENL reaction mediated by antigen-antibody immunological reactions in accordance with the hypersensitivity reaction type III according Comb and Gell (Naaf 2006, Fava, 2012). Good handling on ENL will reduce the number of disability. ENL may arise before and during treatment, even in the completion of treatment (Sampaio, 2000; Naaf, 2006).

At the time of ENL reaction there was an increase in Transforming growth factor (TGF- $\beta$ ) serum, interferon gamma (INF- $\gamma$ ), and interleukin-10 (IL-10), IL-6, IL-8 and IL-1B. While IL-4 and IL-5 remained unchanged (Yamamura, 1992). TGF- $\beta$  is a product of the macrophages that have been activated and the most interesting cytokine because it has a large immune regulatory function and doubles acts (Goulart, 2000). TGF- $\beta$  can regulate a variety of immune cells such as lymphocytes, macrophages and dendrites cells. TGF- $\beta$  has a strong immunosuppressive effect on B cells, T cells CD41, T cells CD81, APC and macrophages (Gorelick, 2002)

In leprosy patients who treated with Multi Drugs Therapy (MDT) will lead the *M leprae* experiencing fragmented, one of the bacteria that is part of that Phenolic-glycolipid-1 antigen (PGL-1). The PGL-1 antigen will stimulate the formation of IgM anti PGL-1 antibodies, this antibody reacts with newly formed PGL-1 antigen and this will cause an ENL reaction (Rojas et al, 1997). Acute symptoms found in ENL are suspected to sign the increasing natural excessive immune function, reflected by an increase in TNF- $\alpha$ , TGF- $\beta$  and IgM anti PGL-1 antibody.

The presence of IgM anti PGL-1 antibodies related to the number of bacteria in *M.leprae* (BI) patients, at the time of ENL reaction the levels of IgM anti PGL-1 antibody are also increased (Stevani 1998). Examination of IgM anti PGL-1 may also be useful as a determinant of early diagnosis and prognosis of leprosy. (Moura, 2008)

The main treatment of ENL reaction is corticosteroids, where the majority of the provision in the long term can reach 2-3 months. Steroids 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 (Kar, Sharma, 2010). In fact, giving steroids in high doses for a long time will cause a lot of side effects and complications (Leal, 2003).

This study is aimed to find out whether the MB type leprosy patients who often suffered from recurrent ENL reactions during the treatment with corticosteroids have a correlation with the levels of TGF- $\beta$  cytokine and IgM antibody anti PGL-1 in the patients serum.

## MATERIALS AND METHODS

This is a cross-sectional comparative study which is aimed to determine the relationship of serum levels of TGF- $\beta$  and IgM antibody anti PGL-1 with the incidence of recurrent ENL reaction. The subjects were leprosy patients at Dr. Abdul Moeloek hospital Lampung and Dr. Rivai Abdullah Leprosy hospital Palembang, aged between 18-60 years and clinically and laboratory determined based on WHO standards (2000), have received treatment with corticosteroids for 2 months and are not suffered from lung tuberculosis and diabetes mellitus, and are not pregnant and lactating for female patients. Both groups were examined their serum levels of TGF- $\beta$  by ELISA using *Human TGF- $\beta$  Bio legend* kit (USA) and IgM anti PGL-1 antibody with the Laboratory of Leprosy Institute of Tropical Disease kit, Airlangga University Surabaya, Indonesia.

The statistical analysis used t-independent, to know the differences in subject characteristics between groups with the recurrent ENL reaction and unrecurrent ENL reaction, Anova was done to know the difference between TGF- $\beta$  and IgM antibody anti PGL-1 in the subject of research with recurrent ENL reaction and unrecurrent ENL reaction, if the distribution is normal, but if it is not followed by Shapiro Wilk test. This research was approved by the Ethics Committee of Medical Faculty, Lampung University, Indonesia.

## RESULTS

The number of subjects were 44 patients consisted of 22 leprosy patients (16 male, 6 female, mean 34,9) with recurrent ENL reaction, and 22 patients (15 male, 7 female, mean 47) of unrecurrent ENL reaction, between ages of 18- 60 years (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
<b>Age group</b>						
< 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	
<b>Gender</b>						
Male	16	72.7		15	68.2	
Female	6	27.3		7	31.8	

**Table 2: Test of Normalitas**

Tests of Normality			
	Shapiro-Wilk		
	Statistic	Df	Sig.
TGF- $\beta$	.953	44	.070
PGL1	.250	44	.000

From the normality test it was obtained p value = 0.200, showing TGF- $\beta$  data has a normal distribution ( $p > 0.05$ ) but IgM anti PGL-1 antibody obtained p value = 0.000. This means that data of the IgM anti PGL-1 was not normal. After conducting data transform, IgM anti PGL-1 has a normal distribution with a significance of  $p = 0.15$  ( $p > 0.05$ ) (table 2).

From table 3 the results of the serum level's test of TGF- $\beta$  by ELISA in patients

with recurrent ENL reaction obtained mean 62.6 (SD 30.4) with the largest concentration of 134.5 pg / ml and the smallest of 23.3 pg / ml and patients unrecurrent ENL reaction obtained mean 47.2 (SD 23) with the largest concentration of 88 pg / ml and the smallest of 11,6 pg / ml.

**Table 3: Descriptif Statistic ENL by ELISA**

ENL	Mean	Standard Deviation
<b>Recurrent</b>		
TGF-b	62,6	30,4
IgM anti PGL-1	2029	1687
<b>Unrecurrent</b>		
TGF-b	47,2	23
IgM anti PGL-1	629	1043

The results of the examination serum levels of IgM anti PGL-1 antibodies by ELISA in patients with recurrent ENL reactions gained mean 2029 (SD 1687) with the largest concentration of 5,704 pg / ml

and the smallest of 150 pg / ml and patients unrecurrent ENL reactions obtained mean 629 (SD 1,043) with the largest concentration of 5.291 pg / ml and the smallest of 125 pg / ml.

**Table 4: Independent sample test**

		t-test for Equality of Means		
		Sig. (2-tailed)	Mean Difference	Std. Error Difference
TGF-β	Equal variances assumed	.015	18.9182	7.4534
	Equal variances not assumed	.015	18.9182	7.4534
IgM anti PGL-1	Equal variances assumed	.000	.57121	.12263
	Equal variances not assumed	.000	.57121	.12633

From table 4 the results of statistic test t-independent, the leprosy group's influence to ENL recurrent reaction and unrecurrent reaction leprosy to the value of TGF-β, it was found that mean p value = 0.015 (p <0.05). It meant that there was a significant difference between the groups of leprosy with recurrent ENL reaction with the unrecurrent reaction one.

The results of the independent t-test statistic, the leprosy group's influence with recurrent ENL reaction and unrecurrent leprosy reaction to the value of IgM anti PGL-1 antibody it was found p value = 0.000 (p<0.05). It means independent-t test the levels of IgM anti PGL-1 antibody has significant difference between the incidence of recurrent ENL reaction and unrecurrent reaction one.

## 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 was unrecurrent 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% (Pocatera, 2006). 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 (Kumar, 2004).

Based on the age of the group with recurrent ENL reaction of most age was 21-30 years 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% (Manandhar, 1999), 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.

### 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 (Yamamura, 1992).

In this study of 22 patients, with recurrent ENL reaction, we obtained levels of TGF-β mean 62, 6 (SD 30, 4), at the laggest 134, 5 pg / ml and the smallest 23, 3 pg / ml, in contrast to the control group, with unrecurrent reaction obtained mean 47 (SD 21, 6) with the laggest levels of 88 pg / ml and the smallest levels of 11, 6 pg/ml. It

is clear that the higher the levels of TGF- $\beta$  in a patient's serum, the more vulnerable the patient to get recurrent reaction of ENL.

This study was the same as that obtained by Goulart (2000), where the level of TGF- $\beta$  in leprosy patients with ENL reaction is higher than those without reaction. TGF- $\beta$  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 (Goulart, 2000). According to Kahawita (2008), there are several pieces of evidence for increased T-cell activity in LL patients with ENL in comparison with patients LL and without ENL. Gorelick (2002) in his study concluded that TGF- $\beta$  mediates the inhibition of T cell differentiation into type 1 Th cell.

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

### **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 (Naaf 2006, Fava, 2012). At the time of ENL reaction, there was increasing of antigen derived from a large number of fragmented bacteria *M. leprae* and reacted with antibodies in the body, there was also a decrease in the function of T suppressor cells (Kahawita, 2008), 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 (Rojas et al, 1997).

In this study of 22 patients, with recurrent ENL reaction, we obtained the levels of IgM anti PGL-1 antibody mean 2,029 (SD 1,687), at the laggest 5,702  $\mu$ /ml and the smallest 23.3  $\mu$ /ml, in contrast to the

control group, with unrecurrent reaction obtained mean 629 (SD 1,043) with the laggest levels of 5, 291  $\mu$ /ml and the smallest levels of 125  $\mu$ /ml .

It is clear that the higher the levels of IgM anti PGL-1 antibody in the serum of the leprosy, the more vulnerable the patient to get recurrent ENL reaction, in this study, it was found a great standard deviation levels of IgM anti PGL-1 antibody, due to the wide variety of data, it shows the heterogeneity of data.

Other studies such as Rojas et al (1997) found that the IgM anti PGL-1 antibody increased in leprosy with ENL reaction compared with unreacted ENL, as well as Moura et al (2008) research which conclude that IgM anti PGL-1 antibody can be a predictor of reaction .While Silva et al (2007) research, getting no differences in levels of IgM anti PGL-1 antibody in leprosy patients with ENL reaction or no reaction. Research Zenha et al (2003) found that a level of IgM anti PGL-1 antibodies in leprosy patients is higher in patients who have not got treatment than those already treated.

Statistical calculation of p value = 0.000 (p <0.005), it means there is a significant difference levels of IgM anti PGL-1 antibodies in leprosy patients with recurrent ENL reaction compared with unrecurrent reaction.

### **CONCLUSION**

In this study, the levels of TGF- $\beta$  and the levels of IgM antibodies anti PGL-1 have significant difference in the incidence of leprosy patients with recurrent ENL reaction than unrecurrent reaction. Increased levels of TGF- $\beta$  and the levels of IgM antibodies anti PGL-1 in patients with leprosy type MB can be a predictor of reaction.

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