The Relationship Between Transforming Growth Factor-β with Erythema Nodosum Leprosum Recurring Events Based on Immunoglobulin-M Anti Phenolic Glycolipid-1 and Cortisol

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ABSTRACT

Background: Erythema Nodosum Leprosum (ENL) recurrent 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. ENL can cause deformity and disability that make the quality of life decreased. TGF-β and IgM anti PGL-1 usually increase during recurrent ENL reaction. Objective: This study was to examine the relationship between TGF-β with recurrent ENL based on the IgM anti PGL-1 and Cortisol on leprosy patients in the Dr.H. Abdul Moeloek Hospital Lampung and Dr. Rivai Abdullah Leprosy Hospital Palembang. Subjects: There were 22 subjects leprosy patients with recurrent ENL reactions and 22 subjects unrecurrent reaction as control. Methods: This research examined the subject serum level of TGF-β by ELISA using Human TGF-β Bio legend kit (USA), IgM anti PGL-1 with the Laboratory of Leprosy Institute of Tropical Disease kit, Airlangga University Surabaya, and Cortisol using Stress Xpress Cortisol EIA kit (StressMarq Canada). Results: This research was from 44 subjects of leprosy MB type, there was 22 subjects with recurrent ENL reaction (16 male, 6 female, mean age 34.9 years old). The mean of TGF-β was 62.6.

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 (9). 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 prostagland in 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 its original dose (11). In fact, giving steroids in high doses for a long time will cause a lot of side effects and complications (12).

Corticosteroids in large dosage and long period could trigger the network corticosteroid insufficiency. Cortisol is a major corticosteroids secreted by the adrenal cortex. In healthy people, without stress, cortisol is secreted by the diurnal variation under the influence of corticotropin released by the pituitary gland. In circulating cortisol binds to globulin. In inflammatory states free corticosteroid levels increasing, this is caused by the release of corticosteroid-binding globulin by neutrophils that plasma cortisol levels down. Exogenous corticosteroids may also suppress the production of corticotropin hormone and corticotropin-relasing and can trigger adrenal atrophy which may persist for months after discontinuation of corticosteroid therapy. (13)

This study is aimed to find out relationship TGF-β with recurrence of ENL based on IgM antibody anti PGL-1 and Cortisol.

MATERIALS AND METHODS
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), IgM antibody anti PGL-1 with the Laboratory of Leprosy Institute of Tropical Disease kit, Airlangga University Surabaya, Indonesia and Cortisol using Stress Xpress Cortisol EIA kit (StressMarq Canada).

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

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 unrecurrent ENL reaction, between ages of 18-60 years old (mean 34.9 ± 13.6) where more males than females with a ratio of 7:3 (table 1).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Recurrent</th>
<th>Unrecurrent</th>
<th>Total</th>
<th>%</th>
<th>mean±SD</th>
<th>Total</th>
<th>%</th>
<th>mean±SD</th>
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<tbody>
<tr>
<td>Age group</td>
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<td>1</td>
<td>34.9 ± 13.6</td>
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<td>4.6</td>
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<tr>
<td></td>
<td>21-30</td>
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<td>31.8</td>
<td>3</td>
<td>13.6</td>
<td>7</td>
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<td></td>
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<tr>
<td></td>
<td>31-40</td>
<td>5</td>
<td>22.7</td>
<td>7</td>
<td>31.8</td>
<td>5</td>
<td>22.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>&gt;50</td>
<td>4</td>
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<td>5</td>
<td>22.7</td>
<td>4</td>
<td>22.7</td>
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</tr>
<tr>
<td>Gender</td>
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<td>72.7</td>
<td>15</td>
<td>68.2</td>
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</tr>
<tr>
<td></td>
<td>Female</td>
<td>6</td>
<td>27.3</td>
<td>7</td>
<td>31.8</td>
<td>6</td>
<td>31.8</td>
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</table>

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 lepra we found BL type bigger than LL type (63.4%) (table 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 ± 23.2 pg/ml.

<table>
<thead>
<tr>
<th>Characteristic subject</th>
<th>Cases</th>
<th>Control</th>
<th>p value</th>
</tr>
</thead>
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<tr>
<td>BI/MI</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>&lt; 3+/0</td>
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<td>21</td>
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<tr>
<td>≥ 3+/0</td>
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<td>4</td>
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<tr>
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<td></td>
<td></td>
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<tr>
<td>BL</td>
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<td>18</td>
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</tr>
<tr>
<td>LL</td>
<td>8</td>
<td>6</td>
<td>18.2</td>
</tr>
</tbody>
</table>

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

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

This study was approved by the Ethics Committee of the Faculty of Medicine, University of Lampung, Indonesia.
The results of the examination mean serum levels of Cortisol by ELISA in patients with recurrent ENL reactions gained 6.61 ± 1.99 μg/dl with the highest levels of 10.22 μg/dl and the lowest of 0.68 μl/ml and in patients with unrecurrent ENL reactions gained the mean levels 5.07 ± 2.01 μg/dl.(table 3)

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, IgM anti PGL-1 and Cortisol with the incidence of recurrent ENL reaction and unrecurrent reaction (table 3).

The statistical Logistic Binary regression had value p of TGF-β = 0.025, IgM anti PGL-1 = 0.016 and Cortisol = 0.771, this means statistically there is relationship the levels of TGF-β with recurrent ENL reaction based on antibody IgM anti PGL-1, whereas cortisol with p> 0.05, meaning that statistically Cortisol is not proven to be variable between TGF-β in the incidence of recurrent ENL reaction (table 4).

Table 4. The statistic Logistic Binary regression

<table>
<thead>
<tr>
<th>Variabel</th>
<th>Df</th>
<th>p</th>
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<tr>
<td>TGF-β</td>
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</tr>
<tr>
<td>IgM anti PGL-1</td>
<td>44</td>
<td>0.016</td>
</tr>
<tr>
<td>Cortisol</td>
<td>44</td>
<td>0.711</td>
</tr>
</tbody>
</table>

**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 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. Semi-quantitative 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-1β. 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 phagocytes 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.

**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 with p> 0.05, meaning that statistically Cortisol is not proven to be variable between TGF-β in the incidence of recurrent ENL reaction (table 4).
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 ± 1687 μg/ml, with the highest levels of 5.702 μg/ml and the lowest levels of 3.687 μg/ml, but in the control group that was unrecurrent reaction with a mean of 629 ± 1043 μg/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.

**Recurrent ENL relationship with Cortisol**

Cortisol is a hormone homeostasis whose function fix imbalance disorder, both derived from a biological organism itself and in the face and adjust to the environment, (22,23).

In the lepra was chronic proinflammatory cytokine release. Increased production of IL-6 which lasted chronic it will inhibit the release / production of ACTH, whereas TNF-α suppress the synthesis of cortisol adrenal through the barriers in the action / work ACTH and angiotensin II in the cells of the adrenal so it caused a decrease in levels of the network (22).

In this study of 22 patients who experience a recurrent ENL reaction, we obtained serum levels of Costisol with mean 6.61 ± 1.99 μg/dl, and the highest levels for 10.22 μg/dl and the lowest levels of 0.68 μg/dl, but in control group that was unrecurrent reaction with a mean of 5.07 ± 2.02 μg/dl. It is clear that the lower levels of Cortisol in the body of the leprosy, the more vulnerable the patient to experience recurrent ENL reactionin this study.

In this study, level of cortisol in leprosy patients with recurrent ENL reaction and unrecurrent mostly below the normal rate is 1-10 mcg /dl. Why cortisol level in the study subjects decreased or lower than the normal value, it was probably because of the patient had long experience of leprosy and ENL reactions and has been receiving treatment with corticosteroids. Each patient in the study had a number of episodes of ENL different, so the corticosteroid dosage received by each profile patients were varied.

According to Van Veen (2009) ENL haunting recurrent and chronic and require long-term treatment. On ENL repeated exposure and lasts a long time, the body experiencing an adrenal fatigue, due to cytokine stimulation which not increased the amount of cortisol to suppress inflammation (24).

Statistical calculation of p value = 0.035 (p <0.05), it means there was significantly difference level of Cortisol in leprosy patients with recurrent ENL reaction compared with unrecurrent reaction.

**The Relationship TGF-β With ENL Recurring Events Based On Ig-M Anti PGL-1 and Cortisol**

The examination of the relationship of TGF-β with the incidence of recurrent ENL is based on IgM anti PGL-1 and Cortisol then performed statistical tests Binary Logistic Regression.

The results of the test Binary Logistic for TGF-βp-value = 0.25, IgM anti PGL-1 = 0.016 and Cortisol = 0.771. This means that IgM anti PGL-1 is statistically proven significant variables between TGF-β in the incidence of recurrent ENL reaction, whereas cortisol with p=0.05, meaning that statistically Cortisol is not proven to be variable between TGF-β in the incidence of recurrent ENL 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.

**REFERENCES:**


