

# The relationship between transforming growth factor- $\beta$ with erythema nodosum leprosum recurring events based on immunoglobulin M anti-phenolic glycolipid-1 and cortisol

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## Abstract

**Objective** To examine the relationship between transforming growth factor (TGF)- $\beta$  with recurrent erythema nodosum leprosum (ENL) based on the anti-phenolic glycolipid (PGL)-1 IgM antibody and cortisol in leprosy patients.

**Methods** This research included 44 patients of leprosy MB type; 22 leprosy patients with recurrent ENL reactions as subjects and 22 patients with nonrecurring reaction as controls. We examined the serum levels of TGF- $\beta$  by ELISA using Human TGF- $\beta$  Bio legend kit (USA), anti-PGL-1 IgM antibody with the Laboratory of Leprosy Institute of Tropical Disease kit, Airlangga University Surabaya, and cortisol using Stress Xpress Cortisol EIA kit (StressMarq Canada).

**Results** There were 22 subjects with recurrent ENL reaction (16 male, 6 female, mean age 34.9 year). Mean TGF- $\beta$  was  $62.6 \pm 30.4$  pg/ml, the IgM anti PGL-1 was  $2029 \pm 1687$   $\mu$ g/ml, and the cortisol was  $6.61 \pm 1.99$   $\mu$ g/dl, and 22 subjects nonrecurring reaction as controls (15 male, 7 female, mean age 47 year) the mean of TGF- $\beta$  level was  $47.2 \pm 23$  pg/ml, anti PGL-1 IgM antibody was  $629 \pm 1043$   $\mu$ g/ml and the cortisol was  $5.07 \pm 2.01$   $\mu$ g/dl. The t-independent statistic test the influence of leprosy MB type group to the recurrent ENL reaction and the un-recurrent ENL patients to TGF- $\beta$ , IgM anti-PGL-1 and cortisol had  $p$  value = 0.015, 0.001 and 0.035 ( $p < 0.05$ ), respectively implying significant difference between subjects and controls. The result of logistic binary test of TGF- $\beta$ , IgM anti-PGL-1 and cortisol was 0.25, 0.016 and 0.771. This means that IgM anti-PGL-1 has correlation as variable of TGF- $\beta$  with recurrent ENL.

**Conclusion** There was significant difference between the levels of TGF- $\beta$ , anti-PGL-1 IgM antibody and cortisol in leprosy patients with the recurrent ENL reaction and those with nonrecurring ENL reaction. There was a correlation between the levels of TGF- $\beta$  with recurrent ENL based on the IgM anti-PGL-1. The increasing levels of TGF- $\beta$  and IgM anti-PGL-1 level on patients with leprosy MB type can be a predictor of the recurrent reaction.

## Key words

ENL, TGF- $\beta$ , IgM anti-PGL-1, cortisol, leprosy, erythema nodosum leprosum.

## Introduction

Erythema nodosum leprosum (ENL) recurrence is a serious complication of leprosy immunology that causes inflammation of the skin, nerves and other organs. ENL can cause deformity and disability that decrease the quality of life. It is

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caused by the deposition of the *Mycobacterium leprae* antigen and antibody complex,<sup>1</sup> type III hypersensitivity reaction according Comb and Gell.<sup>2,3</sup> Good handling of ENL will reduce the incidence of disability. ENL may arise before and during treatment, even in the completion of treatment.<sup>2,4</sup>

At the time of ENL reaction, there is increase in serum transforming growth factor (TGF- $\beta$ ), interferon gamma (INF- $\gamma$ ), interleukin-10 (IL-10), IL-6, IL-8 and IL-1B levels. While IL-4 and IL-5 remained unchanged.<sup>5</sup> TGF- $\beta$  is a product of the macrophages that have been activated and the most interesting cytokine because it has a large immunoregulatory function.<sup>6</sup> TGF- $\beta$  can regulate a variety of immune cells such as lymphocytes, macrophages and dendritic cells. TGF- $\beta$  has a strong immunosuppressive effect on B cells, T cells CD41, T cells CD81, antigen-presenting cells (APC) and macrophages.<sup>7</sup>

In leprosy patients who receive multidrug therapy (MDT) treatment, there is fragmentation of *M. leprae*, and one such component phenolic-glycolipid antigen-1 (PGL-1) is released from bacterial cell wall. The PGL-1 antigen stimulates the formation of IgM anti-PGL-1 antibody, which reacts with newly formed PGL-1 antigen and causes an ENL reaction.<sup>8</sup> Acute symptoms found in ENL are suspected to indicate the increasing natural excessive immune function, reflected by an increase in TNF- $\alpha$ , TGF- $\beta$  and antibody IgM anti-PGL-1.

At the time of ENL reaction, the levels of IgM anti-PGL-1 antibody are also increased. Hence, the examination of IgM anti-PGL-1 antibody may be useful as a determinant of early diagnosis and prognosis of leprosy.<sup>10</sup>

The main treatment of ENL reaction is systemic corticosteroids, used for 2-3 months. Steroids work by inhibiting the inflammatory processes

in early phase and late-phase, decreasing neutrophil chemotaxis and inhibiting prostaglandin synthesis. Once the ENL reaction improves and cures, steroid administration dose can be reduced or discontinued. At the time of dose reduction, ENL reactions often recurs so that the steroid dose should be administered again in original dose.<sup>11</sup> Long-term, high-dose steroids may cause a lot of side effects and complications including hypothalamus-pituitary-adrenal axis suppression.<sup>12</sup>

Cortisol is the major corticosteroid secreted by the adrenal cortex. In healthy people, without stress, cortisol secretion shows diurnal variation under the influence of corticotropin released by the pituitary gland. In circulation, cortisol binds to globulin. In inflammatory states, free corticosteroid levels are increased, secondary to release of corticosteroid-binding globulin by neutrophils that plasma cortisol levels down. Exogenous corticosteroids may also suppress the production of corticotropin and corticotropin-releasing hormone and can trigger adrenal atrophy which may persist for months after discontinuation of corticosteroid therapy.<sup>13</sup>

This study is aimed to find out relationship TGF- $\beta$  with recurrence of ENL based on serum IgM anti-PGL-1 antibody and cortisol.

## Methods

This study was a cross-sectional comparative study, to determine the relationship of serum levels of TGF- $\beta$  with the incidence of recurrent ENL reaction based on IgM anti-PGL-1 antibody 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 and diagnosed clinically and laboratory based on WHO standards,<sup>14</sup> who had received treatment with corticosteroids for 2 months and did not

suffer from lung tuberculosis and diabetes mellitus, and were not pregnant and breast-feeding for female patients. Both groups were examined for their serum levels of TGF-β by ELISA using Human TGF-β Biologend kit (USA), anti-PGL-1 IgM antibody with the Laboratory of Leprosy Institute of Tropical Disease kit, Airlangga University Surabaya, Indonesia and cortisol using Stress Xpress Cortisol EIA kit (StressMarq Canada).

Independent t test was used to know the differences in TGF-β, anti-PGL-1 IgM antibody and cortisol levels between groups with the recurrent ENL reaction and nonrecurrent ENL reaction. Test of logistic binary regression was applied to determine the relationship between TGF-β on the incidence of recurrent ENL reaction based on anti-PGL-1 IgM antibody and cortisol.

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

**Results**

The study population consisted of 44 multibacillary leprosy patients, 22 patients with recurrent ENL reaction as subjects and 22 patients of nonrecurrent ENL reaction as controls. They were 18-60 years old with mean

age of 34.9 ± 13.6 years. There were more males than females with a ratio of 7:3 (**Table 1**).

Bacterial index (BI) in patients with recurrent ENL reaction was <3+ in 16 (72.8%) patients and ≥3+ in only 6 (27.2%) patients, but in control group, most of them (21 patients, 95.4%) had BI of <3+. Regarding type of leprosy, BL type more common than LL (63,4%), (**Table 2**).

The mean serum level of TGF-β by ELISA in patients with recurrent ENL reaction was 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 nonrecurrent ENL reactions had the mean levels of 47.2 ± 23 pg/ml.

The results of mean serum level of anti-PGL-1 IgM antibody by ELISA in patients with recurrent ENL reactions was 2.029 ± 1.687 µg/ml with the highest levels of 5.702 µ/ml and the lowest of 150 µg/ml. In patients with nonrecurrent ENL reactions had mean level of 629 ± 1043 µg/ml.

Mean serum levels of cortisol in patients with recurrent ENL reaction was 6.61 ± 1.99 µg/dl with the highest levels of 10.22 µg/dl and the lowest of 0.68 µg/dl whereas in patients with nonrecurrent ENL reaction the mean level was 5.07 ± 2.01 µg/dl (**Table 3**).

**Table 1** The Characteristics of recurrent ENL reaction by age and gender.

| Variable         | ENL reactions |             |         |           |                |         |         |
|------------------|---------------|-------------|---------|-----------|----------------|---------|---------|
|                  | Total         | Recurrent % | Mean±SD | Total     | Nonrecurrent % | Mean±SD |         |
| Age group (year) | <20           | 4           | 18.2    |           | 1              | 4.6     |         |
|                  | 21-30         | 7           | 31.8    |           | 3              | 13.6    |         |
|                  | 31-40         | 5           | 22.7    | 34.9±13.6 | 7              | 31.8    | 47±18.4 |
|                  | 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** 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    |       |

**Table 3** Mean levels of TGF-β, anti-PGL-1 IgM antibody and cortisol of the study population.

| Variabel               | Group            | Mean  | SD    | Min  | Max   | p value |
|------------------------|------------------|-------|-------|------|-------|---------|
| TGF-β (pg/ml)          | Recurrent ENL    | 62.6  | 30.4  | 23.3 | 134.5 | 0.015   |
|                        | Nonrecurring ENL | 47.2  | 23    | 10.7 | 88    |         |
| IgM anti PGL-1 (µg/ml) | Recurrent ENL    | 2.029 | 1.687 | 150  | 5,702 | 0.001   |
|                        | Nonrecurring ENL | 629   | 1.043 | 125  | 5,241 |         |
| Kortisol (µg/dl)       | Recurrent ENL    | 6.61  | 1.99  | 0.68 | 10.22 | 0.035   |
|                        | Nonrecurring ENL | 5.07  | 2.01  | 0.62 | 8.30  |         |

**Table 4** Logistic binary regression analysis.

| Variable       | Df | P value |
|----------------|----|---------|
| TGF-β          | 44 | 0.025   |
| IgM anti-PGL-1 | 44 | 0.016   |
| Cortisol       | 44 | 0.711   |

Independent t test showed a significant difference between serum levels of TGF-β, anti-PGL-1 IgM antibody and cortisol between subjects with recurrent ENL reaction and controls with nonrecurring reaction (*p* value of 0.015, 0.001 and 0.035, respectively), (**Table 3**).

The logistic binary regression had *p* value of 0.025 for TGF-β, *p*=0.016 for anti-PGL-1 IgM antibody and *p*=0.771 for cortisol, i.e. there is relationship of TGF-β levels with recurrent ENL reaction based on anti-PGL-1 IgM antibody, 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**).

## Discussion

**Age and gender** In the present study, there were 22 MB leprosy patients as subjects who got recurrent ENL reaction and 22 patients with type MB who did not get ENL reaction as controls. There were 16 (72.8%) males and 6 (27.2%)

females in the subject group, while the control group comprised of 15 males and 7 females. This figure is similar to the study from India, where the distribution of the male gender in patients with ENL amounted to 74.2%.<sup>15</sup> It can be explained because the MB leprosy patients are the most vulnerable to ENL. In female with MB type leprosy, pregnancy and childbirth is the originator of ENL reactions.<sup>16</sup>

Regarding the age group, most of the patients with recurrent ENL reaction i.e. 7 (31.8%) belonged to 21-30 year, while in the control group majority i.e. 7 (31.8%) were of 31-40 years age.

The majority of leprosy patients who develop ENL lies in the age range under 40 years; the prevalence in India for patients with ENL under 40 years is 84%.<sup>17</sup> While treating patients with MB leprosy in the age group under 40 years, one should anticipate ENL reaction.

**Bacterial Index** In this study, bacterial index of

<3+ was seen in 72% patients with type MB leprosy who got 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 *M. leprae* in the patient's body is reflected by the index measuring the bacteria.<sup>18</sup>

**Recurrent ENL relationship with TGF- $\beta$**  At the time of ENL reaction there were also increases in serum TGF- $\beta$ , interferon gamma (INF- $\gamma$ ), interleukin-10 (IL-10), IL-6, IL-8 and IL-1B, while IL-4 and IL -5 remained unchanged.<sup>5</sup>

In this study of 22 patients, who experienced recurrent ENL reaction, the highest levels of TGF- $\beta$  at a level of 50-100 pg/ml were seen in 10 (45.5%) patients, with a mean of  $62.6 \pm 30.4$  pg/ml, in contrast to the control group, with nonrecurring reaction, the highest level of < 50 pg/ml was seen in 12 (54.6%) patients with a mean of  $47 \pm 21.6$  pg/ml. It is clear that the higher the levels of TGF- $\beta$  in a patient's body, the more vulnerable the patient to experience repeated reaction of ENL will occur. Our results are similar to those observed by Goulart *et al.*<sup>6</sup> where the level of TGF- $\beta$  in leprosy patients with ENL reaction was higher than those without reaction. TGF- $\beta$  primes macrophages to express inflammatory gene products in response to particulate stimuli, so enhancing the inflammatory response. According to Kahawita *et al.*<sup>19</sup> there is substantive evidence for increased T-cell activity in LL patients with ENL in comparison to LL patients without ENL. Gorelick *et al.*<sup>7</sup> in their study concluded that TGF- $\beta$  mediates the inhibition of T cell differentiation into type 1 Th cell. In the present study, there was a significant difference of TGF- $\beta$  levels in LL patients with recurrent ENL reaction as compared to those with nonrecurring reaction ( $p = 0.015$ ).

**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.<sup>2,3</sup> At the time of ENL reaction, there is increased release of antigen derived from a large number of dead bacteria *M. leprae*, which react with antibodies in the body. There is also a decrease in the function of T suppressor cells.<sup>16</sup> PGL-1 antigen stimulates the formation of anti-PGL-1 IgM antibody, which reacts with newly formed PGL-1 antigen and causes ENL reaction.<sup>8</sup>

In this study, in 22 patients who experienced recurrent ENL reaction, we noticed serum levels of anti-PGL-1 IgM antibody with mean  $2029 \pm 1687$   $\mu\text{g/ml}$ , with the highest levels of  $5.702$   $\mu\text{g/ml}$  and lowest levels of  $150$   $\mu\text{g/ml}$ , but in control group with nonrecurring reaction mean level of  $629 \pm 1043$   $\mu\text{g/ml}$  seen ( $p = 0.001$ ). It is clear that the higher the levels of anti-PGL-1 IgM antibody in the body of the leprosy, the more vulnerable the patient to experience recurrent ENL reaction.

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

**Recurrent ENL relationship with cortisol** Cortisol is a very important hormone of homeostasis which functions to fix imbalance, originating from biological organism itself and

its environment.<sup>22,23</sup> In the leprosy, a number of proinflammatory cytokine are released. Increased production of IL-6 inhibits the release/production of ACTH, whereas TNF- $\alpha$  suppresses the synthesis of cortisol by adrenals.<sup>22</sup>

In this study, in patients who experienced a recurrent ENL reaction, we obtained serum levels of cortisol with mean level of  $6.61 \pm 1.99$   $\mu\text{g/dl}$ , and the highest levels of  $10.22$   $\mu\text{g/dl}$  and the lowest levels of  $0.68$   $\mu\text{g/dl}$ , but in control group with nonrecurring reaction, a mean level of  $5.07 \pm 2.02$   $\mu\text{g/dl}$  was seen. There was significantly difference level of Cortisol in leprosy patients with recurrent ENL reaction compared with nonrecurring reaction ( $p = 0.035$ ).

In this study, levels of cortisol in leprosy patients with recurrent ENL reaction and nonrecurring were mostly below the normal range of  $1-10$   $\mu\text{g/dl}$ . Why cortisol levels in the study subjects were lower than the normal range. The reason might be that leprosy patients with recurrent ENL reactions had been receiving treatment with corticosteroids, which may suppress endogenous cortisol reaction. Each patient in the study had a number of episodes of ENL different, so the corticosteroid dosage received by each patient were varied.

According to Van Veen *et al.*<sup>23</sup> (2009) ENL is a recurrent and chronic problem which requires long-term treatment. With repeated ENL episodes, the body experienced adrenal fatigue, due to cytokine stimulation for increased cortisol release to suppress inflammation.<sup>23</sup>

#### **Relationship of TGF- $\beta$ with recurrent ENL based on anti-PGL-1 IgM antibody and cortisol**

The relationship of TGF- $\beta$  with the incidence of recurrent ENL and anti-PGL-1 IgM antibody and cortisol was performed by using binary logistic regression. The results of this test

showed  $p$  value of 0.25 for TGF- $\beta$ , 0.016 for anti-PGL-1 IgM antibody and 0.771 for cortisol. This means that anti-PGL-1 IgM antibody is statistically significant variable between TGF- $\beta$  and the incidence of recurrent ENL reaction, whereas cortisol is not significant variable between TGF- $\beta$  and the incidence of recurrent ENL reaction

#### **Conclusion**

There was a significant differences between the levels of TGF- $\beta$ , anti-PGL-1 IgM antibody and cortisol between leprosy patients with the recurrent ENL reaction to the nonrecurring ENL reaction. There was a correlation between the levels of TGF- $\beta$  with recurrent ENL based on the anti-PGL-1 IgM antibody level. The increasing levels of TGF- $\beta$  and anti-PGL-1 IgM level in patients with leprosy type MB can be a predictor of reaction.

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