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Research Article

Correlation of Transforming Growth Factor Beta Levels with Bacterial Index on Recurrent Erythema Nodosum Leprosum Reactions

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Abstract

Aim: To analyze the correlation between TGF-β serum levels and bacterial index on leprosy patients with recurrent ENL reaction.

Subjects and methods: Observational analytical study with a cross-sectional approach on 31 subjects aged ≥ 18 years with recurrent type 2 leprosy reactions who sought treatment at the Dermatology and Venereology Policlynic, Chairuddin P. Lubis USU General Hospital, Dr. Pirngadi General Hospital, and H. Adam Malik General Hospital Medan. ENL reccurent patients who were uncooperative, with infectious, autoimmune, malignant diseases, or who were pregnant or breastfeeding were excluded. Basic data recording and subject examination included calculating the bacterial index of leprosy patients using a slit skin smear to measure TGF-β levels. The correlation between TGF-β and the bacterial index was analyzed using the Spearman correlation test. Data processing was carried out with the help of statistical software with a significance value <0.05.

Results: A total of 31 reccurent ENL patients were studied. The majority of recurrent ENL subjects were aged 18 years–25 years and 26 years–35 years, 10 patients each (32.3%), male gender, 21 patients (67.7%), the highest level of education was secondary education, 26 patients (83.9%) and the majority did not work as many as 20 patients (64,5%). Most of the research subjects experienced recurrent ENL reactions during MDT as many as 21 patients (67.7%), with the majority of subjects having BI +2 which is 10 patients (32.2%), and the mean value of TGF-β levels in all subjects was 305.12 ± 45.77. There was a weak negative correlation between TGF-β levels and bacterial index in recurrent ENL subjects (p=<0.05, r=-0.390).

Conclusion: There is a weak negative correlation between TGF-β levels and the bacterial index in recurrent ENL patients.

Keywords: Transforming growth factor beta; Erythema nodosum leprosum; Recurrent erythema nodosum leprosum; Bacterial index; Leprosy reaction

Introduction

Leprosy, also known as Hansen's disease, is a chronic granulomatous infection caused by the bacteria *Mycobacterium leprae* and Mycobacterium lepromatosis. This bacteria is acid-fast and mainly attacks the skin and peripheral nerves [1,2]. WHO data shows that in 2020 there were 127,558 new cases of leprosy worldwide. Indonesia ranks 3rd in the number of leprosy patients in the world, with more than 10,000 cases, after Brazil and India [3,4]. According to 2021 Indonesia Ministry of Health data, 9,061 new leprosy cases were discovered in Indonesia in 2020 [5].

The leprosy reaction known as Erythema Nodosum Leprosum (ENL) or type 2 leprosy reaction is an immunological reaction in leprosy that causes inflammation of the skin, nerves, and other organs. This reaction is a type III hypersensitivity reaction that results in a humoral immune response and consists of a reaction between *M. leprae* antigen and antibodies (IgM, IgG) to form an Antigen-antibody complex (Ag-Ab). This reaction is the most common type of leprosy reaction [6].

The clinical symptom that often occurs in ENL is the appearance of erythematous nodules that are bilateral and symmetrical in distribution, especially on the lower limbs, face, arms, and thighs. These subcutaneous nodules are painful and can ulcerate. Signs of systemic involvement that can appear in an ENL reaction are fever, inflammation of the lymph nodes, neuropathy, and joint, testicular, ocular, liver, and kidney involvement [7].

Transforming growth factor beta (TGF-β) is a multifunctional cytokine that plays an important role in various cellular activities of the human body, as a signal in the cell cycle and the immune system [8,9]. Research from

Petito et al. reported that *M. leprae* itself can induce the production of TGF-β by infecting macrophages, thereby reducing macrophage activity and destroying bacteria [10]. This condition increases the proliferation of *M. leprae* and induces lepromatous leprosy, thereby increasing the possibility of recurrent ENL [11]. Increased TGF-β levels during an ENL reaction can cause greater proliferation of germs and lower elimination of *M. leprae* bacteria, which increases the possibility of recurrent ENL [12].

The Bacterial Index (IB) is an average value that shows the number of bacilli on the skin of leprosy patients. The bacterial index is obtained by adding up the bacilli from each skin scraping site and then dividing it by the number of skin scraping sites [12]. A high bacterial index indicates a high number of *M. leprae* in the body and reflects the host's immune reaction [13,14].

TGF-β and IB levels have been shown to have an important relationship in the pathogenesis and type of leprosy [11,13]. During the development of the immune response, as soon as *M. leprae* enters macrophages, *M. leprae* induces the production of tumor necrosis factor-alpha (TNF-α) and TGF. -β via infected macrophages. Transforming Growth Factor-β (TGF-β) acts as a cytokine suppressor which will inhibit the work of macrophages thereby increasing germ proliferation which is explained by the high IB [15].

In leprosy, patients with recurrent ENL reactions, not many studies have looked at the relationship between TGF-β levels and bacterial indices. This is what prompted the authors to conduct further research regarding the correlation of TGF-β levels with bacterial indices in the reactions of recurrent ENL patients.

Research Design and Methods

Patients and study design

This was a cross sectional with a consecutive sampling methods with sampling method using non-probability sampling that involving 31 patients at Chairuddin P. Lubis USU General Hospital, Dr. Pirngadi General Hospital, and H. Adam Malik General Hospital Medan from June 2022 to November 2023. The inclusion criteria in the research sample were MB-type leprosy patients who experienced active recurrent ENL reactions, aged ≥ 18 years and were willing to take part in the study by signing an informed consent. Exclusion criteria include pregnant and breastfeeding patients, having malignant/autoimmune diseases/other accompanying infections based on the results of the history taking. This study was approved by the Research Ethics Commission of the Faculty of Medicine Universitas Sumatera Utara and Universitas Sumatera Utara General Hospital with a number 364/KEPK/USU/2023.

Methods

This study was conducted at Chairuddin P. Lubis USU General Hospital, Dr. Pirngadi General Hospital, and H. Adam Malik General Hospital Medan. Skin scraping samples were taken from either ear lobes or skin lesions that looked the most erythematous and infiltrative. The bacterial index was calculated by examining the smear results using a light microscope with immersion oil. Blood sample for evaluation of TGF-β levels were carried out at the Integrated Laboratory, Faculty of Medicine, University of North Sumatra, Medan. History taking and physical examination was done. Diagnosis of reccurent ENL was established through history taking and dermatological examination.

Statistical analysis

The correlation of serum TGF-β levels with the bacterial index was analyzed using the Spearman test. The correlation coefficient obtained will be used to measure the strength of the correlation between the two variables. The $p<0.05$ was considered statiscally significant.

Results

The characteristic of reccurent ENL were presented in Table 1. The majority of subjects were men (67.7%) with the largest age groups being 18 years–25 years (32.3%) and 26 years–35 years (32.3%). Based on the latest education level, the majority of subjects had a secondary education level (83.9%) and were not working (64.5%). In addition, the majority of subjects in this study had a Bacterial Index of +2 (32.3%) with a time to recurrent ENL reactions when consuming MDT (67.7%). The average of TGF-β levels in leprosy patients with recurrent ENL reactions in the study had a mean value of 305.12 µg/dL (Table 2).

Table 1: Characteristics of reccurent ENL patients

Characteristics	n	%
Gender		
Male	21	67,7
Female	10	32,3
Age		
18–25 years old	10	32,3
26–35 years old	10	32,3
36–45 years old	6	19,4
46–55 years old	5	16,1
56–65 years old	0	0
Level of education		
Not attending school	0	0
Primary education	5	16,1
Secondary education	26	83,9
Tertiary education	0	0
Jobs		
Doesn't work	20	64,5
Housewife	5	16,1
Employee	3	9,7
Blue-collar workers	3	9,7
Bacterial index		
0	3	9,7
+1	6	19,4
+2	10	32,3
+3	8	25,8

+4	4	12,9
+5	0	0
+6	0	0
Time of onset of recurrent ENL reactions		
Before MDT	0	0
During MDT	21	67,7
After MDT	10	32,3

Table 2: Mean TGF-β levels in recurrent ENL patients

Variable	Mean ± SD	Median (Min-Max)
TGF-β	305,12 ± 45,77	303 (208–428)

Table 3: Correlation of TGF-β levels with Bacterial Index in recurrent ENL

	TGF-β (µg/dL)	Bacterial Index	p	r
Mean (SD)	305 (45,7)	2,12 (1,17)	-0,03*	-0,39*
Median (Min-Max)	303 (208-428)	2 (0-4)	-	-

The relationship between TGF-β serum and bacterial index could also be seen using a scatter plot. The dots on the graph will represent each study subject, with x and y coordinates representing TGF-β serum and bacterial index. By looking at the pattern dots, it can be concluded that there is weak negative correlation between these 2 variable (Figure 1).

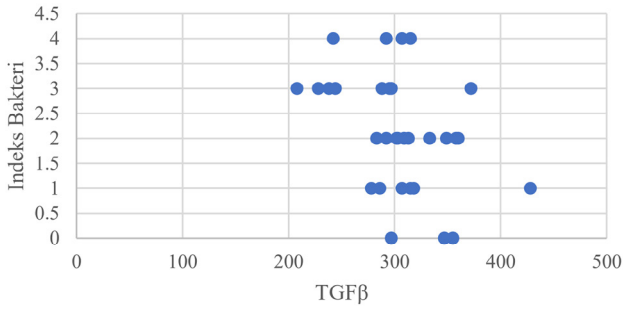

Figure 1: Scatter plot of correlation of TGF-β levels with bacterial index in subjects with recurrent ENL

Discussion

In the research subjects, there were more males, namely 21 patients (67.7%), compared to 10 female patients (32.3%). This is relevant to the research of Pratiwi, et al. and Saraswati, et al. who reported that the distribution of patients with type 2 leprosy reactions in men was greater than in women with a distribution of 74 people (69.8%) and 24 people (68.6%) in each study [16,17]. Melo, et al. reported that the prevalence of ENL was higher in men, namely 17 people (65.4%) and 9 women (34.6%) [18]. In research conducted at RSUP Dr. Soetomo Surabaya by Ditya et al. namely in 2015–2020 it also said that the majority of ENL patients were male, with a percentage of 62.1% for acute ENL and 77.8% for chronic ENL [19].

Type 2 leprosy reactions can affect both men and women. The demographic distribution of the male gender is more common in type 2 leprosy reactions in several studies. However, the explanation of why the male gender is more dominant is still unknown. It is suspected that this is due to the high workload and responsibility of men as the breadwinners of the family as well as the inability to work effectively due to leprosy which is often associated with risk factors for type 2 leprosy reactions to stress [20].

The normality test used is the Shapiro-Wilk test and the results show that the TGF-β data is normally distributed (p=0.37) but the bacterial index is not normally distributed (p=0.025) so the correlation test that will be used is the Spearman correlation test. The results of the Spearman correlation test showed that there was a significant weak negative correlation.

(r=-0.39) between the TGF-β value and the bacterial index (p=-0.03) (Table 3). This means that the research found that increasing TGF-β will tend to reduce the bacterial index value.

The subjects of this study were most often found in the 18 years–25 years old and 26 years–35 years old age groups, namely 10 people each (32.3%) while the fewest were found in the 46 year–55 year old age group, 5 people (16.1%). This is in line with research conducted by Fransisca, et al. which found that 39 people (61.9%) of the type 2 leprosy reaction patients were found to be of productive age [21]. In addition, a retrospective study was conducted at the Leprosy Division of the Skin and Venereology Polyclinic at Dr. Soetomo from 2015 to 2020 found that patients with type 2 leprosy reactions were most often of productive age [22].

One of the risk factors that can trigger a type 2 leprosy reaction, especially recurrent ENL, is productive age. This is related to factors such as productive age and high work activity, which can trigger conditions of stress or physical and mental fatigue [16]. Stress can reduce immunity levels by as much as 50%, which can trigger type 2 leprosy reactions [23].

Stigma affects the psychosocial well-being of leprosy patients. This results in feelings of fear or shame which can lead to anxiety and depression, leading to decreased social participation such as job availability and social exclusion. In anticipation of this stigma, leprosy patients try to hide their condition. This has a negative impact on treatment-seeking behavior in leprosy reaction patients and results in the patient's inability to work as usual [24,25].

In this study, the majority of patients had IB +2 with the highest IB found being +4. This is because the research subjects had recurrent ENL reactions and the subjects had received MDT. One factor that can cause ENL is treatment with MDT, this treatment increases the number of dead bacteria in the patient's body. These dead bacteria function as antigens that stimulate the formation of antibodies, which then combine to form immune complexes. This immune complex then stimulates complement, which

causes ENL [26].

ENL reactions can occur spontaneously before, during, or after the completion of MDT treatment. Several studies show different results regarding the time of onset of ENL. Research conducted by Listiyawati, et al. at Dr. Soetomo Hospital. Surabaya in 2013 showed that the majority of patients (43.8%) experienced ENL after RFT [27]. The emergence of ENL after RFT is more likely to occur in patients with acute ENL, according to most studies due to increased bacterial fragmentation due to MDT treatment which can increase the formation of antigen-antibodies complexes to destroy remaining *M. leprae* antigens and trigger the ENL reaction [19].

Research conducted by Pocaterra, et al. in India revealed that ENL was more frequently found in the third month of MDT therapy than before starting MDT therapy [28]. This was caused by *M. leprae* being destroyed intracellularly by MDT during therapy, causing the bacteria to disintegrate and release antigens. This antigen multiplies so rapidly that it causes the body to respond extraordinarily to get rid of the remaining products of the *M. leprae* bacteria [27].

Based on the results of examining TGF-β levels in leprosy patients with recurrent ENL reactions in this study which are presented in Table 2, the mean value was 305.12 µg/dL. The results of this study are in accordance with the findings of several previous studies on TGF-β expression in ENL. This research is in accordance with research conducted by Rusyani et al. stated that the mean TGF-β level of patients with ENL was 11.7 pg/ml ± 2.7 pg/ml, higher than non-ENL patients, namely 9.5 pg/ml ± 3.6 pg/ml [29].

This study is the first study to correlate TGF-β levels with bacterial index in recurrent ENL reactions. The results of this study are in contrast to the literature which states that

TGF-β plays a role in inhibiting T cell differentiation into Th1 or Th2 subsets through inhibiting GATA-3 expression. Inhibition of differentiation of T cells also directly impacts the release of cytokines which can activate B cells. This results in inhibition of the release of specific antibodies by B cells which will be presented by macrophages. Transforming growth factor-β also has the ability to stop macrophage activity by stopping the production of reactive oxygen and reactive nitrogen factors, which causes infections to become more severe [30,31].

Information regarding the involvement of TGF-β in the pathogenesis of *M. leprae* infection involves the role of the bacteria as an obligate intracellular pathogen in macrophages. The role of TGF-β in suppressing the immune system is demonstrated by an increase in neutralizing anti-TGF-β antibodies in Peripheral Blood Mononuclear Cells (PBMC), this results in increased proliferation and production of IFN-γ from infected cells. Other TGF-β antagonists, including decorin and Latency-associated Peptide (LAP), have also been found to restore lymphocyte responses and IFN-γ production against PBMCs in the late stages of infection [32].

Various components of *M. leprae* have been shown to have the ability to induce TGF-β from patient PBMCs. One of them is LAM which was found to induce TGF-β production from macrophages. The presence of protein and non-protein molecules to induce TGF-β from macrophages will provide an advantage for *M. leprae* [32].

M. leprae infections often develop in individuals with weakened immune systems, such as aging, poor nutrition, or co-infections. In a guinea pig model of protein malnutrition, it was found that macrophages produce increased levels of TGF-β following infection compared with cells from normal control animals, such that overproduction of TGF-β in response to infection may be exacerbated in the presence of protein malnutrition, thereby leading to increased susceptibility to disease [32].

Macrophages that have been infected with *M. leprae* appear to produce active TGF-β which results in infected cells being unresponsive to activation by IFN-γ or TNF-α. Neutralization of TGF-β causes increased bacterial killing upon exposure to cytokines and activated NK cells [32]. The immunosuppressive effect of TGF-β on macrophages, as already described, plays a role in the ability of macrophages to detect phagocytosed bacilli thereby increasing the risk of recurrent ENL [12].

Conclusion

A correlation between serum TGF-β and bacterial index in reccurent ENL patients was observed in this study. Serum TGF-β examination can be used as a biological marker for the inflammatory process in recurrent ENL reactions.

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Conflict of Interest

We declare that, authors have no conflict of interest.

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Author Contribution

All members have contributed equally to the writing of this manuscript.

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