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RESEARCH

TOTAL IGG AND IG ANTI PGL-I WITH DURATION OF THE THERAPY AND REACTIONS IN MULTIBACILLER LEPROSY

(Jumlah Keseluruhan IgG dan IgG Anti PGL-I Mycobacterium leprae dengan Lama Pengobatan dan Reaksi Kusta Multibasiler)

Endang Retnowati[1], Halik Wijaya[1], Indropo Agusni[2]

ABSTRAK

Penyakit kusta masih menjadi masalah kesehatan utama. Penyakit kusta ditandai dengan berbagai spektrum manifestasi klinis dan ragam perbedaan antar spektrum yang ditentukan oleh respons imun dari host. Respons imun humoral di pasien kusta telah dilakukan dengan penelitian mengukur kadar imunoglobulin (Ig). Tujuan penelitian ini adalah untuk mengetahui kenasaban jumlah keseluruhan IgG dan IgG anti PGL-I M.leprae dengan lama pengobatan dan reaksi kusta di pasien kusta tipe MB dengan mempelajarinya. Penelitian dilakukan dari bulan Juni sampai dengan Desember 2013 dengan sampel dari pasien kusta tipe MB di Kabupaten Sampang-Mandura. Serum pasien yang diperiksa adalah jumlah keseluruhan IgG dengan metode Radial Immunodiffusion (RID) dan IgG anti PGL-I M. leprae dengan Enzyme Linked Immunosorbent Assay (ELISA). Data dikumpulkan dan diuji kenasabannya. Median jumlah keseluruhan IgG yaitu 172IU/mL dan median IgG anti PGL-I yaitu 574,33U/mL. Median jumlah keseluruhan IgG di pasien yang menerima pengobatan kurang dari tiga bulan lebih rendah dibandingkan dengan yang lebih dari atau sama dengan tiga bulan. Median jumlah keseluruhan IgG di pasien yang mengalami reaksi lebih tinggi dibandingkan dengan yang tidak mengalami reaksi. Median IgG anti PGL-I di pasien yang sudah diobati kurang dari 3 bulan lebih tinggi dibandingkan dengan yang lebih dari atau sama dengan tiga bulan. Median IgG anti PGL-I di pasien yang mengalami reaksi lebih tinggi dibandingkan dengan yang tidak. Tidak terdapat kenasaban yang bermakna jumlah keseluruhan IgG dan IgG anti PGL-I dengan lama pengobatan dan reaksi kusta pada penelitian ini. Tidak terdapat kenasaban bermakna jumlah keseluruhan IgG dan IgG anti PGL-I dengan lama pengobatan dan reaksi kusta.

Kata kunci: *Jumlah keseluruhan IgG, IgG anti PGL-I, lama pengobatan, reaksi kusta, multibasiler*

ABSTRACT

Leprosy is still a main health problem. Leprosy is characterized by various clinical manifestations and variations between clinical spectra which depend on host immune response. Humoral immune response in leprosy has been studied by examining the immunoglobulin (Ig) level. The purpose of this study is to know the correlation of total IgG and IgG anti PGL-I with duration of therapy and reaction of leprosy in multibaciller leprosy patients.. This study was conducted from June to December 2013 using the samples from multibaciller leprosy patients in the District of Sampang-Madura. The sera were examined for Total IgG with radial immunodiffusion (RID) method and IgG anti PGL-I with enzyme linked immunosorbent assay (ELISA). The data were collected and analyzed for correlation test. The median of total IgG was 172 IU/mL and the median of IgG anti PGL-I was 574.33 U/mL. The median of total IgG in patients with less than three months therapy was lower than the patients who received more than and as equal as three months treatment. The median of total IgG in patients with reaction was higher in reactive than non reactive. The median of IgG anti PGL-I level in patients receiving therapy less than three months was higher than more than or equal as three months groups. The median of IgG anti PGL-I in patients with reaction was higher than in patients with no reaction. There was no significant correlation between total IgG and IgG anti PGL-I with duration of therapy and reaction of leprosy. There was no significant correlation between total IgG and IgG anti PGL-I with duration of therapy and reaction of leprosy.

Key words: Total IgG total, IgG anti PGL-I, duration of therapy, reaction of leprosy, multibaciller

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INTRODUCTION

Leprosy or *Morbus Hansen* is the main chronic infection disease which affects skin, peripheral nerves, upper respiratory and eyes. The causal agent is the acid fast bacteria (ACF), Mycobacterium leprae, which was first identified in 1873 by Gerhard Henrik Armaurer Hansen.[1,2]

Multibaciller leprosy is the source of infection. Leprosy can infect all kinds of people. The *Mycobacterium* can stimulate a specific antibody. Brennan and Brown[3] have discovered a particular *phenolic* antigen called *glycolipid* (PGL)-I from a particular *M. leprae* and used to examine serology in leprosy diagnosis. A high level of IgG and IgM of anti PGL-I is detected in multibaciller patients.[4–8]

The examination of the total IgG by *radial immunodiffusion* (RID) method is easily conducted and does not need sophisticated instruments, while the examination of IgG anti PG-I with *Enzyme Linked Immunosorbent Assay* (ELISA) method needs specific instruments and skilled employees.

Regarding to the above information, this research was conducted in order to analyze the correlation of total IgG and IgG anti PGL-I *M. leprae* within specific duration and leprosy reaction in MB leprosy patients, thus it could help for diagnosis, grouping and management.

METHODS

This research was started from June to December 2013 and it was an analytical observational research which used cross-sectional analysis. The samples were nonrandomly chosen, and serially chosen for samples that fulfilled the criteria until minimal samples were obtained.

The samples were MB leprosy patients in the district of Sampang-Madura, who fulfilled the criteria based on the 1982 WHO grouping according to the 1962 Ridley and Jopling standards

The venous samples were collected from the cubiti vein**,** which was centrifuged immediately at 3000 *rpm* for 5 minutes to obtain the serum. The serum was inserted in *aliquot* tubes, labelled and kept at -70°C in The Tissue Bank Installation of the Dr. Soetomo Hospital, Surabaya until it was examined. The examination of total IgG with RID method was performed in the Clinical Pathology Installation, School of Medicine, Airlangga University -Dr. Soetomo Hospital, Surabaya. The total IgG examination used *NOR Partigen*IgG Siemens Healthcare Diagnostics Products GmbH* 35041 Marburg/Germany by mixing the antisera with agarose gel in a plate /petri dish made of special plastic and the patient's serum which concentration was unknown. The precipitation was shown after an incubation duration of 50–89 hours around the plate as shown in figure 1.

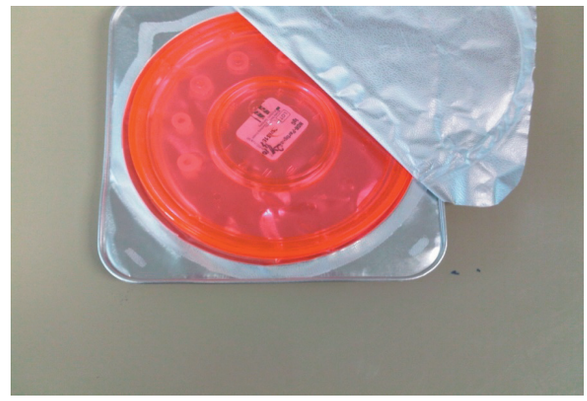

Figure 1. The NOR *partigen plate* for total IgG examination with RID method

The principle of IgG anti PGL-I *M. Leprae* was an indirect quantitative ELISA from the *Leprosy Research Laboratory* in Tokyo, Japan. The examination was done in the *Institue of Tropical Disease* (ITD) Airlangga University, Surabaya. The serum containing antibody will then be determined and bound to the antibody in the solid stage. *Anti human globulin* with enzyme label was added and finally given an additional substrate. Bound enzyme activity was equal to the antibody level in the examined samples.[9–10]

The diagnosis of leprosy which main signs or *cardinal signs* were found in the body such as the presence of skin disorders, such as hypopigmentation, infiltration (skin thickening) erythema (reddish), nodules accompanied by a skin sensitivity disorder, tendency of peripheral nerve thickening, and acid fast bacteria in skin tissue scrapings (positive AFB). The result of total IgG examination with RID method was examined by researchers and skilled laboratory assistants.

A person would be considered as a leprosy patient if there were at least two of the above signs (no. 1–3) or there was a positive AFB.[3] These signs were determined by doctors and skilled medical staffs. A treatment was given by the doctor in charge at that time and given a 3 months treatment based on the program principles.

RESULTS AND DISCUSSION

The number of acceptable samples were 30. The mean age was 35.67, ranged from 13 years to 60 years. The number of male and female patients was

respectively 25 (83.3%) and 5 (16.3%) out of 30 samples.

The comparison between the total male and female patients was 4:1. The mean therapy duration was 2.97 months. The subjects who developed leprosy reaction were 6 out of 30 samples or 20% (see Table 1).

The result of IgG median IgG total in the subjects showed the value range of IgG 53.2–382 IU/mL (see table 2).

The normal value of total IgG was from 700 to 1700 mg/dL or from 80.5 to 195.5 (conversion 1 mg IgG=11.5 IU IgG). The median of total IgG was shown in Table 3.

The therapy duration in this group was divided into two, those who received ones who achieved more than or equal as 3 months treatment. The median of total IgG was higher in the patients who received less than 3 months treatment with a value of 172 IU/mL compared to patients who received more than or equal as 3 months treatment with a value of 176 IU/mL. *Multi Drugs Therapy* (MDT) (a combination of *rifampicin, clofazimine* and *dapsone)* with a median of total IgG 1 based on the absence or presence of leprosy reaction was higher in patients with 207.5 IU/mL reaction compared to patients with no reaction 172 IU/mL (see Table 4).

Table 5 showed the result of IgG anti PGL-I *M.leprae* examination with ELISA method in the patients manifested in media. The median of IgG anti PGL-I *M.leprae* in the subjects was 574.33 U/mL with a range of 30.43–6019.50 U/mL IgG anti PGL-I *M.leprae*.

The median of IgG anti-PGL-I according to the therapy duration (see Table 6) was higher in groups who received less than 3 months therapy with a value of 614.87 U/mL compared to groups who achieved more than or equal as 3 months treatment with a value of 470.15 U/mL. The median of IgG anti PGL-I based on the absence or presence of leprosy reaction was higher in patients with reaction, 904.38 U/mL compared to subjects with no reaction, 520.89 U/mL (see Table 7).

Table 1. The characteristics of research subjects

Sample criteria	Total (n=30)	Percentage (%)
Age (mean±SD)	35.67 ± 14.72 yrs old	
Age range years)		
10–19	4	13.33
20–29	7	23.33
30–39	7	23.33
40–49	2	6.68
50–59	9	30
60–69	1	3.33
Gender		
Male	25	83.3
Female	5	16.7
MDT duration (mean±SD)	2.97±1.99 months	
MDT duration (month)		
<3 months	16	53.3
≥3 months	14	46.7
Leprosy reaction		
Reaction (–)	24	80
Reaction (+)	6	20

Table 2. The examination of IgG total according to RID method

Characteristics	Total IgG (mg/dL)	IgG total (IU/mL)
IgG total level (median)	1395.2	172
Value range	132-3056	53.2 – 382

Table 5. IgG anti PGL-I *M.leprae* examination with ELISA method

Characteristics	IgG anti PGL-I (U/mL)	Cut off (U/mL)
IgG anti PGL-I level (U/mL) (median)	574.33	>610
Value range	30.43–6019.50	

Table 3. The total IgG according to the therapy duration

Characteristics	Case number (n)	Total IgG (IU/mL)	Value interval (IU/mL)	p value
MDT duration (median)				
<3 months	16	172	80.5–243	0.967
≥3 months	14	176	53.2–382	

Table 4. Total IgG based on the presence or absence of leprosy reaction

Characteristics	Case number (n)	Total IgG (IU/mL)	Value Interval (IU/mL)	p value
Leprosy reaction (median)				
Reaction (–)	24	172	132–3056	0.467
Reaction (+)	6	207.5	738–3056	

Table 6. IgG anti PGL-I *M.leprae* according to the therapy duration

Characteristics	Case number (n)	IgG anti PGL-I U/mL)	Value range U/mL	p value
MDT duration (median)				
< 3 months	16	614.87	30.43-6019	0.48
≥ 3 months	14	470.15	75.5-4011	

Table 7. The absence or presence of leprosy reaction IgG anti PGL-I *M.leprae*

Characteristics	Case number (n)	IgG anti PGL-I (U/mL)	Value interval (U/mL)	p value
Leprosy reaction (median)				
Reaction (-)	24	520.89	30.43-5009.7	0.50
Reaction (+)	6	904.38	82.69-6019	

Table 8. Subjects' IgG anti PGL-I *M.leprae* seropositivity

IgG anti PGL-I (>610 U/mL)	Total (n=30)
Seropositive (%)	14/30 (46.67%)
Median (U/mL)	1413.2
Range (U/mL)	629.03- 6019.50

Table 9. The correlation between total IgG and therapy duration and leprosy reaction

Correlation	*R*	*P*
Total IgG vs therapy duration	-0.180	0.340
Total IgG vs leprosy reaction	0.154	0.417

Table 10. The correlation between IgG Anti PGL-I *M.leprae* and therapy duration and leprosy reaction

Relation	*R*	*P*
IgG anti PGL-I vs therapy duration	0.232	0.218
IgG anti PGL-I vs leprosy reaction	0.116	0.543

The seropositive IgG anti PGL-I *M.leprae* was stated if the level of IgG anti PGL-I was above the *cut off* > 610 U/mL. The seropositive IgG anti PGL-I *M.leprae* in 14/30 (46.67%) subjects with median IgG anti PGL-I of 1413 U/mL was found in this research. The details can be seen in Table 8.

The correlation between total IgG and therapy duration was *r* -0.180, and the correlation between total IgG and leprosy reaction was *r*=0.154 (see Table 9).

The correlation between total IgG and therapy duration was *r*=0.232, and the correlation between IgG anti PGL-I and leprosy reaction was *r*=0.116 (see Table 10).

In this research, the median of total IgG level with RID method was 172 IU/mL with a value range of 53.2-382 IU/mL. The level of total IgG in this research was still in the normal range. The examination for healthy people was not conducted, so it was unknown whether or not there was an increase of IgG level in MB leprosy patients. Based on the research conducted by Rawlinson *et al*[11,12], it was stated that there was an increase of IgG level in MB leprosy compared to PB leprosy. MB leprosy had some defects in T lymphocytes, inclusing T *helper* and T *suppressor (Ts) cells*. Ts cells controlled B cell poliferation, so there was uncontrolled B cell poliferation resulting in an increase of IgG in MB leprosy.[12]

The median of total IgG was higher 176 IU/mL in more than or equal as 3 months group than 172 IU/mL in less than 3 months group. The statistical analysis showed that there was no significant difference in less than 3 months groups and more than or equal as 3 months group (*p*=0.967). The leprosy patients who had been treated showed a lot of dead bacteria resulting in fragmentation which entered the blood circulation. The fragmentations were powerful antigenes to raise patient's immune response, such as humoral immunity by producing excessive immune globulins.[13]

The median of total IgG based on the presence or absence of leprosy reaction was higher in the patients with reaction 207.5 IU/mL compared to the patients with no reaction 172IU/mL. Statistically there was no significant difference (*p*=0.467). The number of samples with reaction was 6 patients out of 30 patients or 20%. Leprosy reaction type in this research was type II, *Erythema Nodosum Leprosum* (ENL). Humoral immunity response played a role in this type of reaction. *M. leprae* and other antigenic components which stimulated antibody and producing high imunoglobulin level was not suitable to kill bacteria, but they can bind to antigenes produced by *M.leprae* to form an immune complex. This becomes the basic cause of ENL leprosy.[13] The difference in the various results may be caused by different sample choices because in another research Pausibaciller (PB) and Multibaciller (MB) were used. The method used and the total samples with reaction could be the cause. There were only 6 people (20%) out of 30 patients with reaction in this study.

A lot of *M.Leprae* and other antigenic components were found in Lepromatous Leprosy (LL) and *Borderline Lepromatosus* (BL), so they could stimulate antibody. The high immunoglobulin level is not related to the appropriateness in killing bacilli, but it can stimulate complex immunity. This process becomes the initial cause of leprosy type II (ENL).[14–16]

The median of IgG anti PGL-I *M.leprae* in the subjects was 574.33 U/mL with a value range of IgG anti PGL-I *M.leprae* from 30.43 to 6019.50 U/mL. This value was lower than the number of *cut off* IgG anti PGL-I; this may be due because most patients suffered from leprosy subtype *Mid Borderline* (BB). Silva in 2007[11] reported that the level of PGL-I was higher in MB type leprosy than it was in PB type leprosy.[11]

The median IgG anti PGL-I median was higher, 614.87U/mL in less than 3 months compared to 470.15U/mL in patients treated for more or equal as 3 months. There was no mean difference between IgG and PGL-I level and both based on therapy duration (p=0.48). A research done by Cho in 2001 reported that most of the patients (90%) underwent a decrease of PGL-I level soon after receiving therapy in one month.[17] The decrease in specific IgG *M.leprae* showed that IgG is the sensitive clue for the effect of therapy.[17] In this research, there was no significant difference in less than 3 months group and more than or as equal as 3 months group although there was a decrease of IgG anti PGL-I level. The different result might be caused by the use of distinctive examination methods, IgG anti PGL-I components and *M.leprae* antigenic determinant.

The median of IgG anti PGL-I related to leprosy reaction was higher, 904.38 U/mL when compared to no reaction 520.89 U/mL and was not significantly different (p=0.5).

This result was consistent to the research done by Silva[11] in 2007, that PGL-I antibody was not different from either patients who developed a reaction of leprosy or patients who did not.[11] A research by Andreaoli[18] on 12 patients who developed leprosy reaction type II (ENL) showed that PGI-L antibody towards *M.leprae* in blood circulation decreased and would increase again after the leprosy reaction declined.[18]

IgG anti PGL-I *M.leprae* is considered seropositive if the level of IgG anti PGL-I is above the *cut off* >610 U/mL. Seropositivity in 14 out of 30 subjects or 46.67% of the subjects with a level IgG anti PGL-I of around 629.03 U/mL, and a median of 1413.2 U/mL. The interpretation result of IgG anti PG1-I needed IgM anti PGL-I examination. PGL-I antigen that could stimulate the IgM antibody was found in infected tissues with *M.leprae* and could survive for a long time even after the *M.leprae* germs were dead.

PGL-I antigen can not dissolve in water and can exist in the tissues for a long time by stimulating low antibody responses without living bacilli. Antibody anti PGL-I responses especially in IgM class showed that IgM does not depend on the T lymphocyte cell response towards this glycolipid antigen. It was different from the IgG reponse which pre-dominates major carbohydrate LAM antigen.[17] The correlation of total IgG with the therapy duration resulted in a negative correlation with r value 0.180 (r=-0.180) and p value p=0.340. This means that there was a correlation but not significant between the total IgG level and the duration of therapy in patients.

The correlation of total IgG with leprosy showed a positive correlation with r value 0.154 (r=0.154) and p value p=0.417. This means there was no significant correlation between total IgG and the presence or absence of reaction. The correlation of total IgG and therapy duration showed to be positive with r value 0.232 (r=0.232) and p value p=0.218. This shows that statistically there was a correlation, but not significant between IgG anti PGL-I level and duration of therapy. The same correlation was achieved between IgG level and leprosy reaction, but it showed no significant difference with r value r=0.116 and p value p=0.543. Duthie in 2011[19] reported that IgG anti PGL-I monthly examination in LL leprosy patients after the first MDT showed IgG levels decreasing significantly during therapy.[19] This response of IgG anti PGL-I showed a correlation with bacteria load, clinical picture and grouping after diagnosis, but this was not found in this research. Various possibilities can result in no corrrelation because of the small samples used in this research. It was caused by two differences for choosing samples and only 20% patients who developed reaction, so the obtained data were not homogenous.

CONCLUSION AND SUGGESTION

The total IgG and duration of therapy has no correlation and the total IgG and leprosy reaction show no correlation with IgG anti PGL-I and therapy duration and leprosy reaction. The advantage of this research is that it can contribute to the knowledge about IgG anti PGL-1 (ELISA) and total IgG (RID) examination in leprosy patients.

The examination of total IgG uses an ELISA method. Further research should use a cohort, so that can be found better results.

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