

IgM Anti PGL-1 Antibody Level in Patients with Leprosy: A Comparative Study between Ear Lobes Capillary and Median Cubital Vein Blood Samples

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Abstract

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BACKGROUND: To establish the diagnosis of leprosy accurately, additional examination such as serologic examination with ELISA is required. There are considerations about taking a blood sample from the earlobe region.

AIM: To determine the differences in IgM anti-PGL-1 antibody levels from earlobe capillary and median cubital vein blood sample in leprosy patients.

METHODS: An observational analytic study using a cross-sectional study involving 30 patients with leprosy. ELISA examination of earlobe blood samples with filter paper, and the median cubital vein blood samples with filter paper and conventional methods were performed to determine IgM anti-PGL-1 antibody levels.

RESULTS: The mean value of IgM anti PGL-1 antibody levels from earlobe blood samples with filter paper (1476.62 μ /ml) was relatively similar with median cubital vein blood samples with conventional method (1476.77 μ /ml), but the mean value of IgM anti PGL-1 antibody levels from median cubital vein blood samples with filter paper (1210.37 μ /ml) was lower from other methods. However, there was no statistically significant difference between them.

CONCLUSION: There are no significant differences between the mean levels of IgM anti-PGL-1 antibody from earlobe and the median cubital vein blood samples.

Introduction

Leprosy is a disease caused by *Mycobacterium leprae* (*M. leprae*) that mostly affect peripheral nerve, then skin, mouth mucosal, upper part of the airway, reticuloendothelial system, eyes, muscles, bones and testicle, except central nerve system [1] [2] [3] [4] [5].

According to data from the World Health Organization (WHO) in 2011, there were 210.074 new leprosy cases in the world with a prevalence of 4.06 per 10.000 people. While in the same year, data from the Ministry of Health Republic of Indonesia showed that there were 19.371 new leprosy cases with a prevalence of 8.03 per 100.000 people. In North

Sumatera, there were 170 new leprosy cases with a prevalence of 1.3 per 100.000 people [2] [6] [7].

Leprosy usually diagnosed based on clinical examination, supported by slit skin smear, but in certain cases, additional tests are needed histopathology examination, inoculation on the animal, serologic test and *polymerase chain reaction* (PCR) [8] [9] [10]. Numerous studies have been conducted for these past few years to measure the antibody anti *phenolic glycolipid-1* (PGL-1) using ELISA [8] [9] [10] [11]. There are few problems using blood samples from a median cubital vein for the serologic test because it needs centrifugation and special way of storing and delivering. This process can be difficult to do, especially the peripheral area. Therefore, some studies were done to find solutions for this matter.

A study has been done in detecting IgM antibody against PGL-1 by using filter paper with a *finger prick* method using ELISA test [12]. While in Indonesia, a study was conducted to compare examination of antibody anti-PGL-1 level in blood samples from leprosy patient with and without using filter paper. However, this study showed no significant differences between examination using filter paper and conventional method [13].

Although the explanation on how *M. leprae* could get into the human body is still unknown, some studies showed that it commonly enter through the blistered skin, body parts with low temperature and nasal mucosa [13] [14] [15]. Earlobes have a relatively low temperature, and skin slit smear from earlobes usually shows a positive result for fast acid bacilli, even though it shows negative results in another area [16]. Therefore, we conducted research to compare antibody anti-PGL-1 level in leprosy patient using ELISA test from capillary blood in earlobe using filter paper with blood samples from a median cubital vein with filter paper and the conventional method.

Methods

This research is an observational analytic study with a *cross-sectional* design. It was conducted at Pulau Sicanang Leprosy Hospital, Belawan. The inclusion criteria for this research are a leprosy patient diagnosed based on one of the cardinal symptoms, leprosy patient above 14 years old and willing to participate in this research by signing *informed consent*. The exclusion criterion for this research is if we are unable to take blood smear sample.

The characteristics of research subjects were then compiled and presented according to gender distribution, age group, and type of leprosy. Subjects proceeded to ELISA test using a blood sample from earlobe with filter paper, and blood samples from median cubital vein were also tested using filter paper and conventional method to find out the antibody IgM anti-PGL-1 level. Then it was tested and analysed statistically using ANOVA test to see if there are differences between these methods. If the data is not normally distributed, the statistical analysis will be done using the Kruskal Wallis test. The study was conducted after it was approved by the Research Ethics Commission of the Faculty of Medicine, Universitas Sumatera Utara.

Results

The subject characteristics in this research were showed based on gender distribution, age group and type of leprosy. From a total of 30 subjects, 23 of them (76.7%) were male, and 7 of them were female

(23.3%). This showed that the number of male leprosy patients was more than female. Most patients based on age group is > 45 years old of age group with the total of 12 patients (40%) followed by 15-25 years old of age group with 11 patients (36.7%), and the lowest proportion is 26-35 years old of age group with 2 patients (6.7%). From 30 subjects of research, there were more patients with multibasiler type of leprosy, 28 patients (93.3%) than paucibasiler type of leprosy, 2 patients (6.7%).

Table 1: Characteristics of research subjects

Characteristics	Research subjects	
	n	%
Sex		
Male	23	76.7
Female	7	23.3
Total	30	100.0
Age (year)		
15-25	11	36.7
26-35	2	6.7
36-45	5	16.7
>45	12	40
Total	30	100.0
Leprosy Type		
Paucibasillary	2	6.7
Multibasillary	28	93.3
Total	30	100.0

Different amount of anti-PGL-1 IgM antibody from earlobe capillary blood using filter paper and median cubital vein blood using filter paper and the conventional method.

To compare the amount of anti-PGL-1 IgM antibody from earlobe capillary blood using filter paper with median cubital vein blood using filter paper or conventional method, the scale conversion was made based on regression linear analysis result. The results between this different examination were then analysed using Kruskal-Wallis test due to an abnormality of data distribution.

Table 2: The difference amount of anti-PGL-1 IgM antibody from earlobe blood using filter paper, median cubital vein blood using filter paper and median cubital vein blood using the conventional method on Kruskal Wallis test

		n	p
The amount of anti-PGL-1 IgM antibody	Earlobe blood (filter paper)	30	0.164
	Median cubital vein (filter paper)	30	
	Median cubital vein (conventional method)	30	

Kruskal-Wallis test.

The average amount of anti-PGL-1 IgM antibody from earlobe capillary blood using filter paper (1,476.62 µ/ml) is relatively similar with the average amount of anti-PGL-1 IgM antibody from a median cubital vein using conventional method (1,476.77 µ/ml). However, the average amount of anti-PGL-1 IgM antibody from a median cubital vein using filter paper (1210,37 µ/ml) is lower than both data. After, statistical analysis using the Kruskal-Wallis test, we found that there is no any significant difference between each examination (P = 0.164).

Discussion

From the total number of 30 research subjects, there were more male than a female with most of them aged more than 45 years old and suffered from Multibacillary Leprosy. By data from the Ministry of Health Republic of Indonesia in 2007, most countries in the world, except in some countries in Africa, leprosy is more commonly found in male than female. This probably influenced by environmental or biological factors. Although leprosy can be found at all ages, this disease particularly affected young and productive age people [2]. Furthermore, data from Indonesia in 2011 showed that Multibacillary leprosy cases are higher (15,634 people) than Paucibacillary Leprosy (3,737 people) [7].

Filter paper has been considered to be used for diagnosis of new cases in leprosy. A study showed that there is a straight correlation between antibody anti-PGL-1 test using a blood sample from filter paper and classical method [12]. However, another study in Indonesia found that there are no significant differences between the examination of antibody anti-PGL-1 level using filter paper and conventional method [13]. From our study, we did not find a significant difference between each methods ($P = 0.164$). There are other similar study and it showed that blood samples from skin smear site had a higher level of antibody compared with sera and may be more sensitive for antibodies measurement [16].

Statistic analysis results from the examination of anti-PGL-1 IgM antibody amount from earlobe blood using filter paper, median cubital vein blood using both filter paper and conventional method indicate a similar relative interpretation. However, there are some advantages in taking blood samples from the earlobe, as it can also be taken while obtaining slit skin smear samples for bacteriological examination. Furthermore, special storage and delivery process are not needed. Therefore it is more convenient in the peripheral area.

In conclusion, the study showed similar results between mean antibody IgM anti-PGL-1 level from earlobe capillary blood samples using filter paper and median cubital vein blood samples using the conventional method. However, the mean antibody IgM anti-PGL-1 level from median cubital vein blood samples using filter paper is lower than both data, although the difference is not significant.

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