The correlation of Ig M Anti PGL-1 antibody between blood veins and dryed capillary blood on filter papers in household contact of leprosy patient

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Abstract

Delays of leprosy detection and treatment can lead to disability and potential transmission. Serologic examination has the advantage in detecting Subclinical Leprosy. The procedure of serologic test, which is one of its main limitation, could be simplified by the use filter paper. This study aims to assess the effectiveness of the use of capillary blood dropped on filter paper as a substitute for venous blood in household contact of leprosy patients. Seventeen samples of capillary blood dried on filter paper and venous blood samples from the same individual were examined by ELISA method to determine the levels of IgM anti-Phenolic glycolipid-1 (PGL-1). The mean of anti-PGL-1 IgM levels of filter paper samples 163.31±126.16; whereas the mean of levels from venous samples was 473.16±411.26. There was significant difference and correlation between these two groups. Samples on filter paper in household contact can be used to determine the level of anti-PGL-1 IgM in serum by converting on the regression basis. Further study is required to evaluate the potency of filter paper methods to conduct large-scale serological screening.

Introduction

Leprosy or Hansen's disease is a chronic infectious disease caused by *Mycobacterium leprae* (*M. leprae*). Leprosy can be cured but it still continues to cause significant health problems in many parts of the world. World Health Organization (WHO) reported the number of new cases of leprosy in Southeast Asia in 2015 as many as 154,834 cases where Indonesia ranks third largest after Brazil is as many as 17,025 cases.[1]

Leprosy is still a health problem in Indonesia, including East Java. Transmission of leprosy is still high enough with the incidence rate of new leprosy in East Java which in 2014 found 4,114 cases.[2] In Dr. Soetomo General Hospital, Surabaya, Indonesia, there are 713 (6.49%) new leprosy patients from a total of 10,970 leprosy patients in the Outpatient Clinic 2011-2015.[3]

The failure of leprosy elimination is caused by the failure of the transmission chain termination, namely the difficulty of early detection of Subclinical Leprosy (SL), a condition characterized by specific antibodies against *M. leprae* high enough in the blood without any clinical symptoms. SL is thought to be primarily derived from household contacts of leprosy patients, because until now it is believed that the main transmission of leprosy is from Multi bacillary leprosy patients.[4,5]

Early diagnosis of leprosy is an important issue as late detection and treatment can result in disability as well as the psychosocial impacts due to the persistent stigmatization of the disease in the community. In addition, it is also necessary to watch out for potential transmission of leprosy in cases that are too late to be detected. Various studies on leprosy diagnostic serologic examination continue to be developed. Phenolic Glycolipid-1 (PGL-1) is a specific antigen for *M. leprae* which was first discovered in the 1980s. Ig M anti PGL-1 antibodies indirectly indicate the presence of *M. leprae* in individuals, which may reflect both SL and leprosy. Seropositivity of IgM anti-PGL-1 antibodies was found to be 80-100% in untreated untreated multibasilar (MB) leprosy patients, whereas in patients with pausibasiler (PB) type, there was a lower titer of IgM antibodies with 30-60% seropositivity.[6-8]

Detection of antibodies against PGL-1 has been widely used for community surveys, early diagnosis, treatment monitoring, reaction monitoring and identification of relapses. At this time serology was performed mostly using a blood serum sample from the cubital vein. The main obstacles to this serologic examination in addition to the relatively expensive costs are often a problem in the field associated with fairly complex sample treatments such as: the blood samples taken have to be sent from the field to the central laboratory at a controlled temperature to ensure stability; still need a centrifuge for serum taking; and in certain cases such as children and the elderly, the process of taking blood is difficult to perform. If the time period since the blood sample is taken until the serum is examined in the laboratory long enough, preservatives and special storage are required. All of the above obstacles cause leprosy serology tests are difficult to do on a large scale in leprosy endemic areas. It is well known that the serum of blood required for serological tests does not need too much, just 50 µl of blood serum can be obtained from a few drops of blood. For that we need to develop a more practical blood-taking method that is through filter paper that can absorb the blood and then dissolved by soaking process. Blood storage to filter paper has several advantages such as it does not require complicated sample processing and samples can be stored for long periods so as to facilitate serological examination in leprosy endemic areas that generally have limited health facilities and infrastructure. In addition, blood sampling could derived from an easier location such as from a fingertip.

Prakoeswa in 2013 reported that 6% subclinical leprosy with high IgM anti-PGL-1 titers become manifest leprosy in 4 years, that means 1.5% annually. Range of Ig M anti-PGL-1 antibody titer in leprosy

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patients with seropositive range between 605 units / ml (cut off for population of East Java) up to > 5300 units / ml. While the anti-PGL-1 titer study was conducted in East Java populations divided into seropositive +1 (605-1,000 u / ml), +2 (1,000-2000 u / ml), +3 (2,000-3,000 u / ml) and +4 (> 3,000 u / ml). Individuals with +3 and +4 seropositivity are potentially become manifest leprosy.[9]

Until now there has been no research on the examination of Ig M anti-PGL-1 antibody levels by using capillary blood on filter paper in household contact of leprosy patients in Outpatient Clinic Dermatology Venereology, Dr. Soetomo General Hospital, Surabaya. The aim of this study was to evaluate the effectiveness of the use of capillary blood using filter paper as a substitute for serum samples by proving the presence of correlation between anti-PGL-1 IgM levels in fingertip capillary blood using filter paper with serum IgM anti-PGL-1 serologic level from cubital vein blood of household contact. The benefits of this study were to obtain a diagnostic SL in household contact that was simpler and easier to perform by officers in the field, especially in the endemic area of leprosy.

Materials and Methods

The design of the study was observational cross-sectional analytic. The sample of the study was a household contact of multibacillary leprosy patients who came to the Outpatient Dermatology and Venereology Clinic, Dr Soetomo General Hospital, Surabaya that meets the inclusion criteria of the study: Household contact who live in the house and close contact for at least 6 months with multibacillary leprosy patients (spouses, parents, or children) maximum of 3 domestic people. Blood samples were collected following local ethics committee approval from Dr. Soetomo General Hospital and after participants signed their informed consent forms.

A sample of 10 µL of capillary blood from a vaccinotile / Micro Hematocrit capillary tube/plain (MHC tube) wound on the fingertips was taken by a capillary micro tube containing heparin. The blood is impregnated on Whatman chromatography paper (Whatman International Ltd, Maidstone, UK) filter and dried at room temperature, subsequently labeled and inserted in a plastic bag. After that, it was inserted in eppendorf tube, plus 900 µl dilution buffer (PBRT and Skim milk) and filter paper for 2 hours to get optimum result, followed by Vortex for 5 minutes to dissolve serum. Dilution of 1: 300 with dilution buffer is then subsequently stored at a temperature of 2-10°C until the ELISA test time is carried out. Furthermore, 2 cc of blood samples were taken from the cubital vein of household contact of leprosy patients, centrifuged, serum was taken, preserved, stored at 2-10°C until the ELISA test was performed. IgM anti PGL-1 serology test with ELISA method: a technique to measure anti-PGL-1 IgM antibody level, using synthetic antigen NTP-P-BSA particles which is a combination of synthetic tricaccharides with BSA (Bovin Serum Albumin). This antigen is reacted with domestic serum serum with a certain dilution and is a reaction between specific antibodies (anti PGL-1) with synthetic antigens. The result is a staining reaction read with a spectrophotometer (Elisa reader) with Optical Density (OD) unit at 492 nm. Further data is stored and processed with Biolyse / X read program. The result is quantitative data with unit / mL unit.

Results

Seventeen fingertips blood capillaries samples of household contact were dried on filter paper and 17 cubital vein blood samples from the same individuals were examined by ELISA method to determine the levels of IgM anti PGL-1. ELISA examination revealed the mean of IgM anti-PGL-1 level from capillary blood sample on filter paper was 163.31±126.16; whereas the mean of IgM anti-PGL-1 levels from serum samples cubital vein was 473.16±411.26. There was significant difference between capillary sample and cubital vein (p=0,006). In Figure 1, we can see the difference of IgM anti-PGL-1 levels in capillary blood samples on the filter paper and cubital venous blood samples in each sample where the anti-PGL-1 IgM level in capillary blood samples on the filter paper is lower than the cubital vein blood sample.

Pearson correlation test showed significant correlation between IgM anti-PGL-1 level from capillary blood sample on filter paper and cubital vein blood samples (p=0,00) with Correlation Coefficient 0,994 (strong correlation). Figure 2 shows that the higher anti-PGL-1 IgM level in the cubital vein is followed by the rise of the anti-PGL-1 IgM titres at the fingertips. In the regression calculation, the regression equation was obtained: Y (IgM anti-PGL-1 blood sample from cubital vein) = -7.704+2.944 X

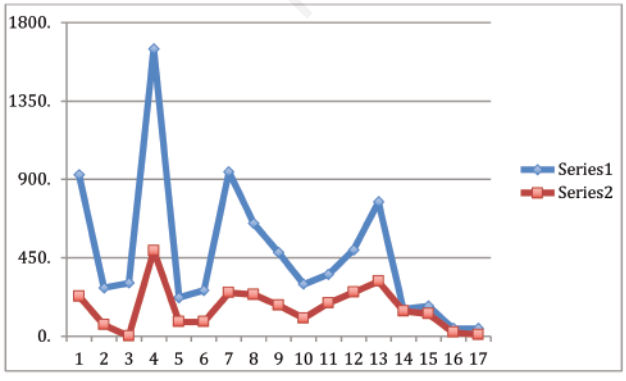

Figure 1. The comparasion titer IgM anti-PGL-1 level from capillary blood sample on filter paper and cubital vein blood samples.

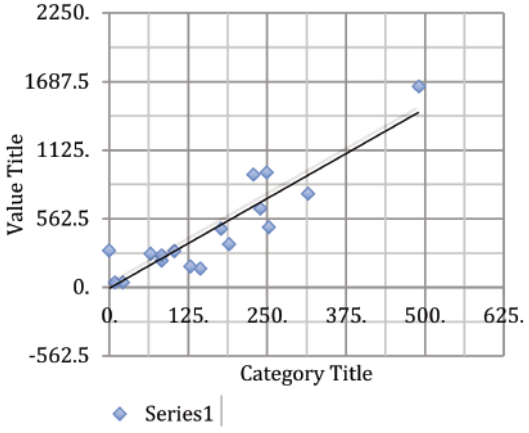

Figure 2. The correlation between titer IgM anti-PGL-1 level from capillary blood sample on filter paper and cubital vein blood samples.

(IgM anti-PGL-1 capillary blood sample on filter paper). Using this formula we can convert the anti-PGL-1 IgM results taken from the fingertip capillary blood using a filter paper to determine the anti-PGL-1 IgM titres in cubital venous blood.

Discussion

Household contact is an important determinant of leprosy transmission chain failure due to potential SL that would potentially be a source of transmission and become manifest leprosy. Detection of SL by using Ig M Anti PGL-1 titer with serum sample has been done by Moura (2008) Furini (2011) Lobato (2011), and Cabral (2013), but not much research has been done for SL detection by using filter paper.[7,10-12] In this study, it has been demonstrated that in household contact of leprosy patients, the Ig M Anti PGL-1 filter paper is significantly lower than that of the anti-PGL-1 Ig M titles. This corresponds to the difference in the process of both methods, namely the use of filter paper obtained less than directly measuring the titer of the serum. The lower yield on filter paper is caused partly by absorption of blood and followed by blood dissolution, so not all samples can be dissolved. A study conducted by Prakoeswa (2007) in leprosy patients showed similar results, Ig M Anti PGL-1 filter paper was significantly lower than that of IgM Anti PGL-1 in serum ($p < 0.05$).[13] Differences between Prakoeswa's research (2007) and this study were the mean of anti-PGL-1 IgM levels from capillary blood samples on filter paper (624,73±493,71) higher than our study (163.31±126.16); similarly, the mean titer of IgM anti-PGL-1 of the serum samples from cubital vein in the Prakoeswa's study (1534,2±1267,95) was higher than the results we obtained (473.16±411.26). This corresponds to the difference in the number of IgM Anti PGL-1 circulating in the patient's blood relative to the contact persons, which is based on the difference in the number of *M. leprae* in the individual body. Syahputra (2003) reported that no significant difference in Ig M anti PGL-1 antibody levels between blood samples taken from cubital vein was then dropped onto filter paper and serum samples of leprosy patients ($p=0.147$).[14] Differences in the results of this study with Syahputra (2003) were caused not only by the different samples used (household contact versus patients), but also on filter paper method, Syahputra using cubital venous blood vein, while our research using fingertip capillaries. The use of filter paper from fingertip capillary drop to replace venous blood should be demonstrated by a strong correlation between them and followed by the determination of the regression equation as we have done, where a strong correlation with 0.941 correlation coefficient is obtained. Research with different sample that is using patient of leprosy done by Prakoeswa (2007) and Yamashita (1999) also showed the same result that is strong correlation with correlation coefficient respectively that is 0.98 and 0.97.[13,15] The advantages of filter paper can be used in areas with high temperatures and humidity as in the tropics, and the result is no change in yields for long enough storage, ie 1 year at -10-4 ° C. Other factors such as temperature, humidity, length and way of storage and sample delivery can be said not to affect the results of the inspection when performed in accordance with the procedure.[15]

Conclusions

This study supports the detection of SL by examination of fingertip capillary blood samples on filter paper because of good results, relatively cheap with simpler methods than cubital vein blood samples. It could facilitate large scale serological screening in the field for detecting SL especially in household contact, early diagnosis of leprosy, monitoring of treatment outcomes and identification of relapse and reactions.

References

1. World Health Organization. Global Leprosy Update 2015: time for action, accountability and inclusion. Weekly Epidemiological Record 2016; 91:405-20.
2. Kementrian Kesehatan RI Direktorat Jenderal Pengendalian Penyakit Dan Penyehatan Lingkungan. Pedoman Nasional Program Pengendalian Penyakit Kusta. Jakarta; 2015.
3. Annual report Outpatient Clinic Dr Soetomo General Hospital, Surabaya. 2016. Surabaya: 2016.
4. Prakoeswa CRS, Agusni I, Izumi S. Detection of Mycobacterium leprae DNA in blood of the subclinical leprosy. Folia Medica Indonesiana 2007; 43(2): 64-67.
5. Prakoeswa CR, Citrashanty I, Astari L, Listiawan MY, Agusni I. Beberapa prosedur diagnostik terkini pada penyakit kusta. Dalam: Prakoeswa CR, Agusni I, Listiawan MY, editors. Kapita selekta penatalaksanaan Morbus Hansen terkini. Surabaya: Dept/SMF Ilmu Kesehatan Kulit dan Kelamin FK Unair/ RSUD Dr.Soetomo Surabaya, 2013. h. 1-25.
6. Spencer JS, Brennan PJ. The role of mycobacterium leprae phenolic glycolipid i (pgl-i) in serodiagnosis and in the pathogenesis of leprosy. Lepr Rev 2011; 82: 344-357.
7. De Moura RS, Calado KL, Oliviera MLW, Bührer-Sékula S. Leprosy serology using PGL-I: a systematic review. Rev Soc Bras Med Trop 2008; 41(Supl 11): 11-18.
8. Britton WJ, Lockwood DNJ. Leprosy. The Lancet 2004; 363: 1209-1219
9. Prakoeswa CRS, Citrashanty I, Astari L,Iswahyudi, Wahyuni R, Adriaty D, Agusni I. Importance of subclinical infection in leprosy control. 12th Asia Pasific Environmental and Occupational Dermatology Symposium (APEODS) in conjuction with 13th Annual Scientific Meeting of Indonesian Society of Dermatology and Venereology (PIT - PERDOSKI).: 2013.
10. Bazan-Furini R, Motta ACF, Simão JCL, Tarquínio DC, Marques Jr W, Barbosa MHN, et al. Early detection of leprosy by examination of household contacts, determination of serum anti-PGL-1 antibodies and consanguinity. Mem Inst Oswaldo Cruz 2011; 106 (5): 536-40.
11. Lobato J, Costa MP, Reis EM, et al. Comparison of three immunological tests for leprosy diagnosis and subclinical infection. Lepr Rev 2011; 82: 389-401.
12. Cabral PB, Cunha JE, Macedo AC, et al. Anti-PGL1 salivary IgA/IgM, serum IgG/IgM, and nasal Mycobacterium leprae DNA in individuals with household contact with leprosy. Int J of Inf Dis 2013; 17: 1005-1010.
13. Prakoeswa CR, Listiawan MY, Adriaty D, Wahyuni R, Iswahyudi, Agusni I, et al. Korelasi kadar antibodi Ig M anti PGL-1 antara darah vena cubiti dengan darah kapiler yang dikeringkan di kertas saring pada penderita kusta. Berkala Ilmu Kesehatan Kulit dan Kelamin 2007; 19(2): 115-119.
14. Syahputra E, Matodihardjo S, Ervianti E, Agusni I, Izumi S. Penggunaan Kertas Saring untuk Uji Serologi Kusta. Maj Kedok Indon 2004; 54 (12): 491-95.
15. Yamashita T, Nguyen H, Maeda M, Flageul B. Anti PGL-1 determination using blood collection on filter paper in leprosy patients. Rev. Inst. Med. Trop. 1999; 41(4): 239–42.