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Research Report

COMPARATIVE STUDY OF FILARIAL DETECTION BY MICROSCOPIC EXAMINATION AND SEROLOGICAL ASSAY UTILIZING BMR1 AND BMXSP RECOMBINANT ANTIGENS FOR EVALUATION OF FILARIASIS ELIMINATION PROGRAM AT KAMPUNG SAWAH AND PAMULANG, SOUTH TANGERANG DISTRICT, BANTEN, INDONESIA

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ABSTRACT

South Tangerang district is one of the endemic areas for filariasis; and based on an evaluation study in 2008-2009 which covered several subdistricts, the prevalence of microfilaria was between 1–2.4%. Nevertheless, the evaluation by serological assay has never been reported. A cross-sectional study was conducted to detect the microfilaremia and anti-filarial IgG4 antibody status in Kp Sawah and Pamulang subdistricts. Cluster sampling was performed in Kp Sawah by collecting finger-prick blood (FPB) and venous blood samples from inhabitants who lived with and nearby the four elephantiasis subjects in the area. The FPB were only collected in Pamulang area by consecutive sampling method. The detection method included microscopic evaluation of FPB and serological detection using recombinant antigens BmR1 and BmSXP by ELISA and lateral flow rapid tests. Symptomatic patients who had 2nd and 3rd degree of elephantiasis were clinically determined in 10% (4/40) subjects. Among those with elephantiasis, 2 were positive serologically but their microscopic results were all negative (40/40). Meanwhile, the microscopic result for 107 subjects from Pamulang were all negative. The results of the rapid tests showed that 15% (6/40) of the positive cases were detected by Brugia Rapid and 27.5% (11/40) by PanLF. Meanwhile, the ELISA showed that 20% (8/40) of the cases were positive with BmSXP, whereas only 2.5% or 1/40 sample was found to be positive with BmR1. Even though the sensitivity of the Rapid test was lower when compared to microscopic examination for these samples, the assay showed good specificity ranging from 72.5 to 97.5%. The optical density (OD) values of ELISA has ranged between 0.3–3.045.

Key words: *Microfilaremia, BmR1, BmSXP, Brugia rapid test, PanLF*

ABSTRAK

Kabupaten Tangerang Selatan merupakan salah satu wilayah endemik filariasis; dan berdasarkan studi evaluasi tahun 2008-2009 yang mencakup beberapa kecamatan dengan prevalensi antara 1–2.4%. Namun demikian, belum ada laporan tentang hasil evaluasi secara serologi. Studi potong lintang dilakukan untuk mendeteksi status mikrofilaremi dan keberadaan antibodi anti-filaria IgG4 di kecamatan Kp sawah dan Pamulang. Pengambilan sampel dilakukan secara Cluster sampling dengan sampel darah jari (SDJ) dan sampel darah vena dari penduduk yang tinggal di sekitar empat penderita elefantiasis di wilayah Kp Sawah. Sedangkan di wilayah Pamulang hanya dilakukan pengambilan darah jari dengan metode consecutive sampling. Metode deteksi dilakukan secara mikroskopis terhadap SDJ dan secara serologi dengan menggunakan rekombinan antigen BmR1 dan BmSXP dengan cara ELISA dan tes cepat Brugia Rapid. Penderita simptomatik yang terdeteksi elefantiasis berjumlah 10% (4/40) diketahui dengan status limfedema ekstremitas derajat 2 dan 3. Diantara penderita elefantiasis tersebut, 2 orang terdeteksi positif secara serologis, namun hasil mikroskopisnya negatif (40/40). Sementara itu, hasil mikroskopis dari 107 SDJ di wilayah Pamulang seluruhnya negatif. Hasil tes cepat menunjukkan 15% (6/40) positif terhadap Brugia Rapid dan 27.5% (11/40) positif terhadap PanLF. Hasil ELISA pada sampel penelitian ini menunjukkan

20% (8/40) positif terhadap BmSXP, namun hanya 2.5% (1/40) yang positif terhadap BmR1. Meskipun nilai sensitifitas tes cepat lebih rendah dibandingkan mikroskopis pada sampel penelitian ini, namun nilai spesifisitasnya tinggi yang berkisar antara 72.5 to 97.5%. Nilai optical density (OD) dari hasil ELISA berkisar antara 0.3–3.045.

Kata kunci: *Microfilaremia, BmR1, BmSXP, Brugia rapid test, PanLF*

INTRODUCTION

Lymphatic filariasis is targeted for the Global Elimination Program initiated by WHO and the program is expected to be successful by 2020. An epidemiological data maps out that until 2008, there are 316 regencies/ municipalities out of 471 regencies/municipalities in Indonesia which have been declared as the endemic areas of filariasis.[1] The South Tangerang regency is one of endemic area for filariasis with a prevalence of microfilaria ranges between 1–2.4% covering several subdistricts as mentioned by an evaluation in 2008.[2]

The Health Department of South Tangerang district in the same period found that the prevalence of filariasis in Ciputat subdistrict has reached 1.6% with 8 patients has clinically suffered from lymphedema or elephantiasis in 2002; while in other subdistricts including Pondok Aren, Setu and Pamulang, the prevalence are 1.8%, 1%, and 2.4%, respectively. An area is defined to be endemic for filariasis when the microfilaria rate has 1% of prevalence.[2] A previous study to evaluate microfilaremia and antigenemia status, which was conducted in Kp Sawah, Ciputat, South Tangerang district in 2012, showed that 5% subjects were positive for microfilaria and 27.5% subjects had positive results for IgG4 antifilarial antibody using rapid test.[2]

There are some factors that may affect the success of filariasis elimination program, i.e. accurate diagnosis and evaluation on the success of continued diagnostic work-up and treatment.[3] Mass Drug Administration for filariasis in South Tangerang district, has been performed annually and been evaluated microfilaremia by using finger-prick blood (FPB) since 2002. Nevertheless, an evaluation by serological assay to detect antigenemia in blood vein has never been reported.

The present cross-sectional study was conducted to identify the microfilaremia and antibody anti-filarial IgG4 status in Kp Sawah (Ciputat) and Pamulang areas. The method included microscopic evaluation for FPB and serological detection using recombinant antigens BmR1 and BmSXP1 for blood vein samples of inhabitants living in Kp Sawah area. Diagnostic tests were also performed to identify the sensitivity and specificity of both antigens in detecting the presence of antibody anti-filarial IgG4 in the blood.

MATERIAL AND METHODS

A cross-sectional study was designed to conduct filariasis evaluation by observational, questionairy, and laboratory methods. Diagnostic tests were performed to detect the presence of microfilaria by microscopic and IgG4 antibody antifilarial by rapid test and ELISA. Samples were collected using cluster sampling technique in Kp Sawah by obtaining samples from some inhabitants who lived nearby the 4 patients who had been diagnosed with elephantiasis in the area. finger-prick blood (FPB) was also collected in West Pamulang area by consecutive sampling; however, the local Health Department advised that the blood vein samples should not be collected at the time. Samples were collected at night (10 pm – 2 am.) as the microfilaria activity in peripheral blood reaches its peak in those hours.

Microscopic examinations were performed at the Parasitology Laboratory Faculty of Medicine and Health Sciences, Syarif Hidayatullah State Islamic University on 40 samples obtained from Kp Sawah and 107 samples obtained from West Pamulang. The FPB were prepared into thick-blood smear slide and subsequently stained using Giemsa staining (Merck©) before they were examined under microscope. The volume of blood for microscopic was 1-2 drop(s) of peripheral blood. The calculation of microfilaria found in FPB was performed using the following formulation:

$$\text{Mf density (mfd)} = \frac{\text{Total number of microfilariae found in the sample}}{\text{Total number of slides with positive Mf}} \times 50^{*}$$

* 50 is the correctional factor for blood volume of 20 µl; while for different blood volume, the correctional factor is also different.[4]

Subsequently, the vein blood was examined using recombinant antigens BmR1 and BmSXP to detect antibody anti-filarial of IgG4 in blood circulation. The serological examination was performed at the laboratory of Institute for Moleculer Medicine (INFORMM) in University Science Malaysia (USM) using both rapid test and ELISA.

The measurement of rapid test was done using recombinant antigens (BmR1 and BmSXP) and the results were characterized by the development of 2-3 strips (bands) indicating positive results or the presence antibody anti-filarial of IgG4 in sample serum. The instrument used for detecting the presence of *Brugia sp* infection is *Brugia Rapid test*; while for detecting *W. Bancrofti,* the ICT

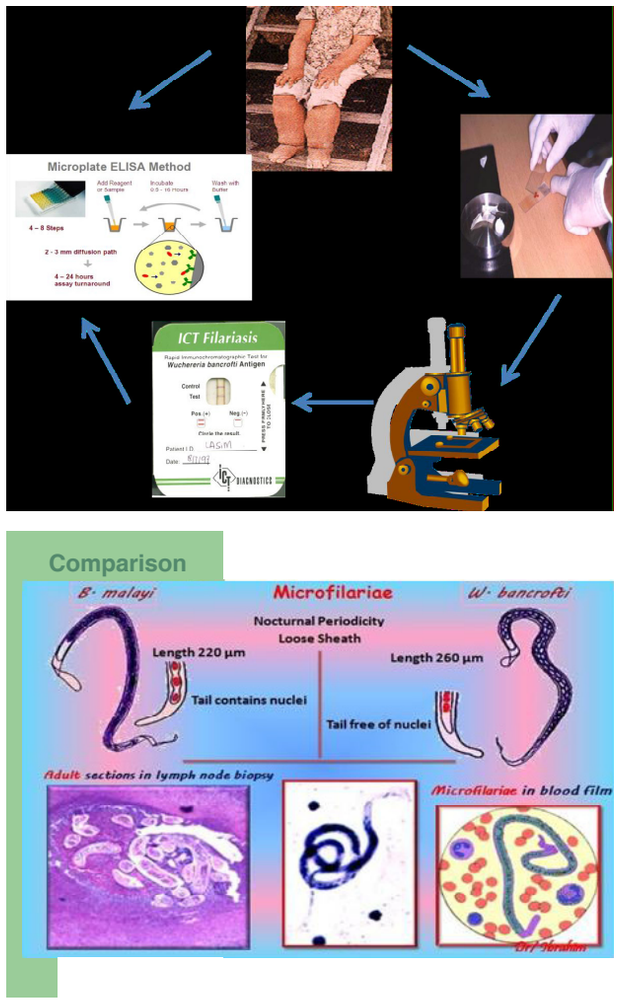

Figure 1. Method of diagnostic stages to detect Lymphatic Filariasis

bancrofti or Pan LF was utilized. (Reszon Diagnostik International. Bhd, Malaysia).

The measurement of IgG4 antibody antifilarial level using ELISA technique was also performed according to the standard procedure at the laboratory of INFORMM-USM (Penang, Malaysia) as mentioned by Rahmah et al (2001a). Each well of the ELISA plate was coated with 100 µL of recombinant antigen BmR1/BmSXP in 20 µg/mL $NaHCO_3$ buffer (pH 9.6).

Conjugates containing monoclonal anti-human IgG4-HRP (Horseredish Peroxidase-CLB Netherland) were inserted in each well of the plate as much as 1: 4500 in PBS. Additional substrate of ABTS (Boehringer Mannheim, Germany) was given for each well after washing and the plate was subsequently covered with aluminium foil and incubated for ½ hour.[5]

The result of reaction was read using ELISA spectrophotometer (Dynatech, USA) at 410 nm wavelength. The measurement results were presented in Optical Density (OD) with a cut-off point of 0.300.[5] Serum sample with OD ≥ 0.300 was categorized as sample with positive IgG4 and those with OD < 0.300 was considered negative.

RESULT AND DISCUSSION

Evaluation on the success of filariasis elimination program was supported by instruments of assay, which had high sensitivity and specificity in detecting the presence of specific infection of filarial species in filariasis endemic area.[4]

The use of recombinant antigens of *BmR1* and *BmSXP* by utilizing rapid test and ELISA had higher sensitivity and specificity compared to using microscopic examination.

Table 1. Results of Diagnostic Test for Samples from Kp. Sawah and Pamulang

Index	Detail from Kp Sawah	Total result Kp Sawah	Result from Pamulang
Microscopic Examination:			Amicrofilaremic: 100% (107/107)
➢ Microfilaremic	0		
➢ Amicrofilaremic	100% (40/40)		
Rapid test	27.5% (11/40)	Rapid test:	No serological test was performed
➢ Positive *PanLF*	15% (6/40)	➢ Positive: 27.5%	
➢ Positive *Brugia Rapid*	15% (6/40)	➢ Negative 72.5%	
➢ Positive *Brugia Rapid & PanLF*	60% (24/40)		
➢ Negative			
ELISA:		ELISA:	
➢ Positive *anti BmXSP1* (OD: 0.3–3.045 or strong positive)	20% (8/40)		
➢ Positive *anti BmR1* (OD: 0.645 or strong positive)	2.5% (1/40)	➢ Positive: 20%	
➢ Positive *W. bancrofti* and *Brugia s*	2.5% (1/40)	➢ Negative: 80%	
➢ Negative	77.5% (31/40)		

It can detect and differentiate filarial infection caused by *Wuchereria bancrofti* and *Brugia sp*, both for individuals with amicrofilaremia and those with microfilaremia. The recombinant antigen is to confirm microscopic result, which have some limitations, i.e. the sensitivity depends on blood volume and parasite periodicity; therefore, it is less sensitive, particularly for individual with symptomatic amicrofilaremia.[6]

Several study report by using microscopic in some endemic filariasis area in South Tangerang and Banten province has found negative result in all slides of FPB. However, serological examination has never been reported for these areas.

An evaluation on filariasis elimination program in the last 5th-annual period in 2013 at Ciputat and Pamulang area reveal the following results:

Symptomatic subjects who had 2nd and 3rd degree of elephantiasis at Kp Sawah in the present study were 4/40 subjects or 10% of all subjects in the study. Among the respondents who had elephantiasis, there were only 2 subjects who had positive serological results for *W. Bancrofti* filariasis, but their microscopic results were all negative; therefore, the inhabitants in the study site could be categorized into:

a. Asymptomatic microfilaremic patient: 0
b. Asymptomatic amicrofilaremic patients: 90% (36/40)
c. Symptomatic microfilaremic patients: 0
d. Symptomatic amicrofilaremic patients: 10% (4/40)

Meanwhile, the microscopic result for 107 subjects from Pamulang were all negative, and there was no blood vein collection nor serological test was performed at the time. Total for microscopic result of 147 samples from the two areas are negative.

The results of the rapid tests showed that 15% (6/40) of the positive cases were detected by Brugia Rapid and 27.5% (11/40) by PanLF. This is not surprising since both recombinants antigens can detect both kinds of filariasis, however BmSXP has greater diagnostic sensitive for bancroftian filariasis while BmR1 is more sensitive in detecting brugian filariasis. Noordin, 2003 has reported that BmSXP antigen showed 91% sensitivity using serum of *W. bancrofti*- infected individuals and 39% sensitivity using serum from brugian filariasis patients.

Meanwhile, the ELISA showed that 20% (8/40) of the cases were positive with BmSXP, whereas only 2.5% or 1/40 sample was found to be positive with BmR1. These results indicated that the study site is endemic for bancroftian filariasis and this idea is supported by the clinical manifestations. The optical density (OD) values ranged between 0.3–3.045. Even though the sensitivity of the ELISA test was lower when compared to microscopic examination, the assay showed good specificity ranging from 72.5 to 97.5%.

The serological diagnostic test can also detect and differentiate infection specifically between *Wuchereria bancrofti* and *Brugia sp* since there is a recombinant filarial antigen of BmR1 and BmSXP1 coated on the rapid test as well as for the ELISA. The results of serological test, which were mostly positive with recombinant antigen of *BmSXP,* indicates that the study site was endemic for Bancrofti and this idea is supported by the clinical manifestations, which revealed the presence of 2nd and 3rd degree of elephantiasis. However, positive result of *Brugia* found by ELISA in one single sample of asymptomatic subject and in 6 samples of PanLF rapid test has indication of possibility for potential transmission of Brugria filariasis in the area.

Appropriate results may become a reference point for evaluation of filariasis program, whether the program is successful or not in the endemic area. It will affect the future policy of filariasis program that should be taken into consideration by the local health department, i.e. whether they will continue the filariasis program or whether it should be stopped. The process of stopping MDA for filariasis is illustrated in the figure below.

CONCLUSION

Serological detection using antigen BmR1 and BmSXP for inhabitants in Kp sawah and Pamulang area shows that infection of filaria *W. bancrofti* and *Brugia sp* is remained endemic. Eventhough sensitivity of the ELISA test was lower when compared to microscopic examination, the assay showed good specificity ranging from 72.5 to 97.5% for the presence of *W. bancrofti* and *Brugia* filaria with titer of IgG4 antifilarial antibody ranging between 0.3–3.045.

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