Lymphatic Filariasis Caused by *Brugia malayi* in an Endemic Area of Narathiwat Province, Southern of Thailand

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Abstract

Lymphatic filariasis caused by *Brugia malayi* is highly prevalent in Narathiwat province of Thailand. The World Health Organization has aimed to eliminate the disease globally by the year 2020. To achieve the goal, assessment of the real disease situation should be integrated as part of the control program. The preliminary data for long-term study of the disease situation in this endemic area is necessary for the elimination program of lymphatic filariasis. By using the conventional microscopic method, the microfilarial rate of *B. malayi* in an endemic area of Narathiwat province was 1.38 per cent. The microfilarial densities ranged from 17 microfilariae/ml to 1,250 microfilariae/ml median = 50. The highest prevalence was found in the age group > 45-60 (4.69%). The lowest microfilarial rate was in the age group ≤ 15 (0.37%). The infection in males was about three fold the number in females. A PCR-based method was employed to detect a *B. malayi*-specific *Hha* I repetitive DNA sequence with high specificity and sensitivity. The PCR assay will be useful in assisting the elimination program of lymphatic filariasis in control and monitoring the disease in Thailand.

Key word : Lymphatic Filariasis, *Brugia mal*ayi, Thailand

TRITEERAPRAPAB S, KARNJANOPAS K, PORKSAKORN C, SAI-NGAM A, YENTAKAM S, LOYMAK S
J Med Assoc Thai 2001; 84 (Suppl 1): S182-S188

Lymphatic filariasis is a mosquito-borne disease caused by filarial nematode parasites, mainly *Wuchereria bancrofti* and *Brugia malayi*. More than 120 million persons are infected with the parasites, with the people at risk of more than 1 billion[1,2]. After being bitten by infected mos-

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quitoes, humans obtain the infective third-stage larvae from the mosquitoes. The larvae migrate through the skin and go to the lymphatics, where they develop into adult worms. After mating, the female will produce microfilariae into the blood circulation. When the mosquito vectors take their next blood meal from the patients, microfilariae will be taken up and develop into the infective stage in the vectors. In infected cases, the host-parasite interactions will lead to chronic pathology including lymphedema, hydrocele and elephantiasis. Therefore, the disease could seriously affect socio-economic status of the endemic areas.

Lymphatic filariasis caused by *B. malayi* is endemic in Asian countries including the South of Thailand, mainly Narathiwat Province(3). The disease has been declared by the international task force to be eliminated by the year 2020(4). To achieve the goal, before the initiation of the national control program in Thailand beginning in the year 2001, close monitoring of the Filariasis control program is necessary. Long-term assessment of the real burden of disease situation in the endemic area should be implemented. This study will serve as preliminary data of the prevalence of brugian filariasis in this endemic area of Thailand before the elimination program is launched. Furthermore, to increase the sensitivity for detection of *B. malayi*, we also set up a PCR-based method using primers specific for *Hha* I repeat sequence.

PATIENTS AND METHOD

Study area

The study was performed in cooperation with health personnel from The Royal Development Project, Phikultorng Center, Narathiwat Province and the Filariasis Division, Department of Communicable Disease Control, Ministry of Public Health. The study area is an endemic area of *B. malayi* infection including Sungai Padee, Tak Bai, Cho I Rong and Sungai Kolok districts, in Narathiwat province. Narathiwat is the southernmost Thai coastal province facing the Thai Gulf and borders northeast Malaysia. The provincial capital is 1,437 kilometers south of Bangkok by road and 1,116 kilometers by train. The province covers some 4,475 square kilometers, two thirds of which are forested mountains. The inhabitants of Narathiwat are largely farmers and fishermen. The area is endemic for lymphatic filariasis caused by *B. malayi*. Narathiwat has an average rainfall of 1,100-4,000 mm/year. The average temperature of this area is 26-28°C. There are plenty of tropical forests and water sources suitable for breeding of mosquito vectors of *B. malayi*(3). The temperature, moisture and several swamps, both open swamp and closed swamp, are suitable for the development of the main mosquito vectors, *Mansonia* sp, of *B. malayi*(5).

Study population

The study population was 2,469 Thais residing in the study area mentioned above. Verbal informed consent was obtained from each individual and child's parents or guardian in the presence of two witnesses. All individuals were well informed about the dangers of filariasis and the disease's consequences. Individuals who were microfilaremic were treated with standard treatment, diethylcarbamazine (DEC), as provided by the health personnel from Filariasis Division, CDC Department, Ministry of Public Health, Thailand.

Detection of *Brugia malayi* microfilariae

Baseline demographic data, previous history of lymphatic filariasis and DEC treatment were obtained from all participants. Duplicate of approximately 60-ul blood samples from finger-prick sterile technique were obtained and smeared onto microscope slides as previously described (6,7). After being air-dried, the blood films were stained with Giemsa's technique. All identified microfilariae were *Brugia malayi* which were counted and the number found in each patient was recorded.

Extraction of DNA

The QIAamp blood method (Qiagen, Chatsworth, CA) was used to extract DNA as described by the manufacturer's protocol.

Polymerase chain reaction conditions

Forward and reverse PCR primers were designed based on the consensus sequence of the *Hha* I repeat(8). The sequences of these primers, which allow amplification of a 322 bp DNA fragment from *B. malayi*, were as follows; forward primer (18-mer):

5'-GCGCATAAATTCATCAGC-3',

reverse primer (23-mer):

Table 1. The prevalence of *Brugia malayi* infection classified by age and sex.

Age group (years)	Male			Female			Total		
	Number of sample	Number infected	%	Number of sample	Number infected	%	Number of sample	Number infected	%
≤ 15	423	2	0.47	396	1	0.25	819	3	0.37
> 15-30	305	5	1.64	379	3	0.79	684	8	1.17
> 30-45	236	3	1.27	281	1	0.36	517	4	0.77
> 45-60	138	10	7.25	139	3	2.16	277	13	4.69
> 60	84	5	5.95	81	1	1.23	165	6	3.64
Total (%)	1,186	25	2.11	1,275	9	0.71	2,462	34	1.38

5'-GCGCAAAACTTAATTACAAAAGC-3'. Reagents for PCR were obtained from Perkin-Elmer Cetus (Norwalk, CT).

PCR reactions were performed using 2 µL of the DNA extracts prepared from blood samples as described above. Reagents were used at the following concentrations in a 50 µL total reaction volume: 10 mM Tris-HCl, pH 9.2, 1.5 mM $MgCl_2$, 75 mM KCl, 0.1 mM of each primer, 0.2 mM of each deoxynucleotide triphosphate, and 2 units of *Taq* polymerase. The temperature programme for the PCR was 35 cycles of 30 sec at 94°C, 30 sec at 55°C, and 1 min at 72°C, and final extension of 10°C min at 72°C. A negative control for the PCR assay using DNA extract from non-infected individuals in the reaction mixture was included with all runs of PCR. A positive control was also included using 10 pg of *B. malayi* genomic DNA.

Analysis of the polymerase chain reaction products

Ten µL of the PCR products were loaded on to 1.5 per cent agarose gel and a unique band of 322 bp was visualized using an ultraviolet transluminator after ethidium bromide staining.

Data analysis

Data were recorded and analyzed by using Microsoft Excel version 6.0.

RESULTS

Characteristics of study population in Narathiwat Province

A total of 2,462 individuals participated in this study (Table 1). There were more females (1,275; 52%) than males (1,186; 48%). Most were in the young age group; 33 per cent (819) were less than 15 years old (36% for males and 31% for females). There were 28 per cent (684), 21 per cent (517), 11 per cent (277), and 7 per cent (165) in the age groups > 15-30, > 30-45, > 45-60, and > 60, respectively.

Microfilaremia classified by age and sex

The prevalence of brugian filariasis as assessed by microfilaremia was 1.38 per cent (34 cases; 25 males and 9 females) (Table 1). The microfilarial densities ranged from 17 microfilariae/ml to 1,250 microfilariae/ml with the median of 50. The prevalence was lowest in the youngest age group (0.37%; 3 cases) and increased to the peak at > 45-60 age group (4.69%; 13 cases). Then the prevalence declined to 3.64 per cent (6 cases) at the age group > 60. More men were infected with *B. malayi* than women. The ratio of infection in males to females was 2.8 : 1.

PCR for *Hha* I repeat sequence

A PCR-based assay to detect specific *Hha* I repeat of *B. malayi* has been developed to identify infected cases with high sensitivity and specificity[8-11]. However, this sensitive and specific method has not been employed to evaluate the disease burden in Thailand. Therefore, we set up a PCR-based system for detection of *B. malayi Hha* I repeat. Our results showed that the test could detect as little as 10 fg of genomic DNA from adult *B. malayi* (Fig. 1). The PCR assay could detect the microfilaremic cases. Fig. 2 shows an example of *Hha* I repeats amplified from DNA extraction of blood from seven microfila-

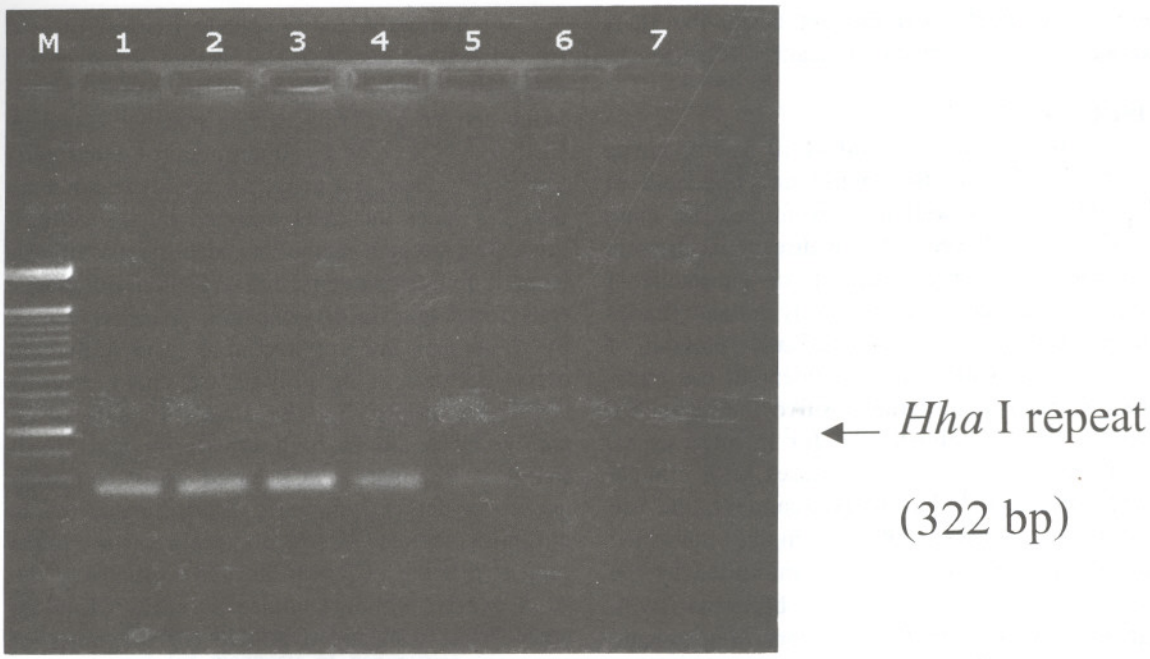

Fig. 1. Sensitivity of a PCR assay to detect *Hha* I repeat DNA (322 bp) by using specific primers described in Materials and Method. Lane M: 100 bp marker; Lane 1-6: adult *B. malayi* genomic DNA 100 pg, 10 pg, 1 pg, 100 fg, 10 fg and 1 fg, respectively; Lane 7: negative control.

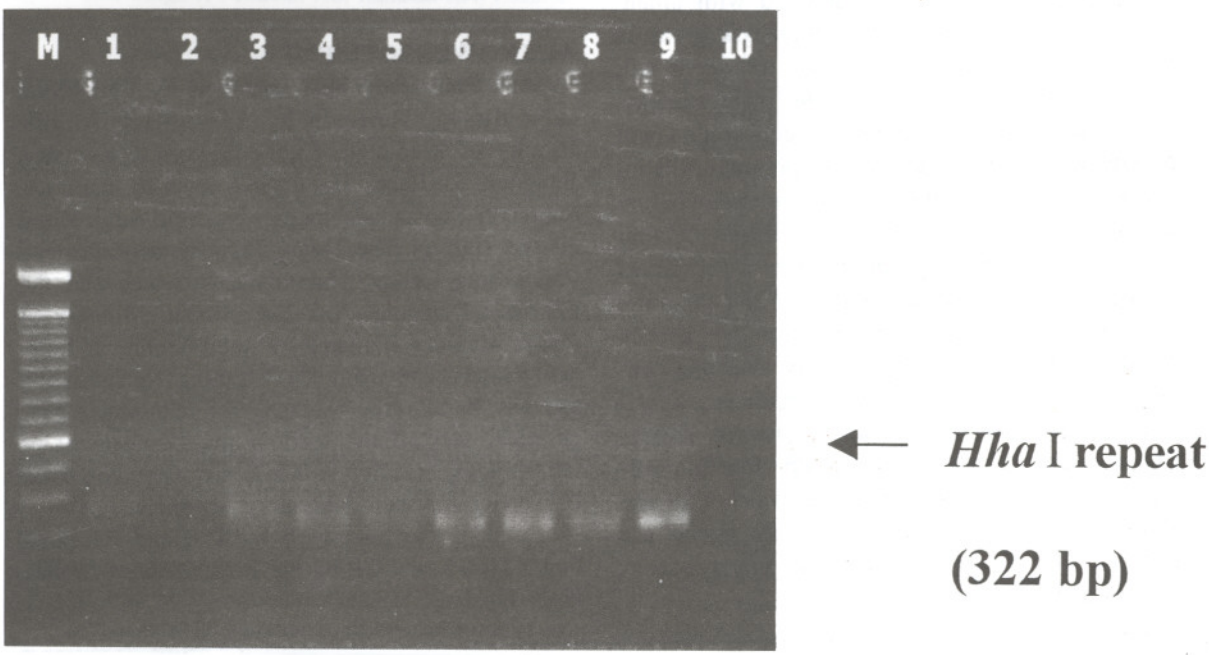

Fig. 2. Detection of *Hha* I repeat DNA (322 bp) by a PCR assay using specific primers described in Materials and Method. Lane M: 100 bp marker; Lane 1: positive control using 10 pg *B. malayi* genomic DNA; Lane 2: negative control; Lane 3-9: *Hha* I repeats amplified from blood samples of seven microfilaremics; Lane 10: negative control.

remics. The PCR assay did not detect the DNA extracted from the blood of healthy volunteers.

DISCUSSION

Basically, the control of vector-borne diseases include vector control and treatment of infected cases as well as prevention. The main strategy for each vector-borne disease is different from each other, which depends on the nature of each pathogen and vector. For lymphatic filariasis, the main strategy of the elimination program of the disease includes mass treatment in the transmission area (microfilarial positive rate $\geq$ 0.2%), selective drug administration for infected cases, and rehabilitation in chronic cases with clinical manifestations[12]. For mass treatment, the use of diethylcarbamazine (DEC) (6 mg/kg) combined with albendazole (400 mg), recommended by the World Health Organization, has been used with high effectiveness for the treatment of lymphatic filariasis[13,14]. The high microfilarial rate (1.38%) in our study area compared with that reported previously is an indicator for mass treatment in this area[3,12]. As part of the elimination program, Thailand will launch a national control program using DEC combined with albendazole for mass treatment in high risk areas in the year 2001. The annual mass treatment will continue for 5 years. Besides the main strategy, health education and community cooperation will be emphasized. Furthermore, the program will include evaluation of the disease situation, before and after the program implementation, together with close monitoring and interim assessments of the input, process and output of the program. However, to detect *B. malayi* infection, we, here in Thailand, still have to use the only available conventional microscopic method, which is insensitive. The procedures may fail to identify amicrofilaremics or individuals with very low microfilaria levels. To assess the real prevalence of the disease, highly sensitive and specific assays are required to evaluate the lymphatic filariasis control program[7].

Currently, for lymphatic filariasis caused by *Wuchereria bancrofti*, the highly sensitive and diagnostic method is detecting the specific circulating antigen of the parasite in patients' blood by ELISA[7,15-17] or by immunochromatographic test[18-20]. Both antigen tests could detect more than 2-3 times the cases detected by the conventional microscopic method to identify microfilaria from blood. However, such effective diagnostic tests for diagnosis of lymphatic filariasis caused by *B. malayi* are not available. The molecular diagnostic test using polymerase chain reaction (PCR) by amplifying the *Hha* I repeats of *B. malayi* DNA shows high sensitivity and specificity[8]. Although a PCR-based assay is mainly useful to detect lymphatic filarial parasites in mosquito vectors,[21-24] our preliminary results suggest that the PCR can be used to diagnose the active cases who are microfilaremic while no other better tests are available for diagnosis of *B. malayi*. We have set up the PCR assay of *Hha* I repeat sequence that can detect as little as 10 fg of *B. malayi* genomic DNA. The application for use in field studies should be useful for the national control programme of lymphatic filariasis in Thailand.

ACKNOWLEDGEMENT

This study was supported by The Molecular Biology Research Fund, Research Affairs, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand. The authors wish to thank Dr. Saravudh Suwannadabba for his kind advice and all the staff at The Royal Development Project, Phikultorng Center, Narathiwat Province and the Filariasis Division, CDC Department, Ministry of Public Health, Thailand for their technical help. We also wish to thank Dr. Issarang Nuchprayoon and Ms Narak Triteeraprapab for manuscript preparation, and all the staff at the Department of Parasitology, Faculty of Medicine, Chulalongkorn University for their laboratory assistance.

ST was supported by Thailand Research Fund (TRF), and CP by the Royal Golden Jubilee Ph.D. Fund.

(Received for publication on March 21, 2001)

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