

***Wuchereria bancrofti* -specific circulating antigen for diagnosis of bancroftian filariasis**

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- Background** : Routine diagnosis of *Wuchereria bancrofti* by microscopic examination of microfilariae from night blood is time-consuming, and has low sensitivity. A better diagnostic test is required for accurate estimates of the prevalence and to evaluate the control program of bancroftian filariasis.
- Objective** : To evaluate the sensitivity and cross-reactivity of the *Wuchereria bancrofti* -specific Og4C3 antigen-capture assay for diagnosis of bancroftian filariasis.
- Setting** : Mae Sot district, Tak Province.
- Design** : Cross-sectional study.
- Subjects** : Ninety-five Myanmar migrants who were microfilaremic. There were 22 males and 73 females.
- Methods** : Each participant was examined for microfilariae by microscopy for Og4C3 antigens and anti-filarial IgG4 antibodies by enzyme-linked immunosorbent assays (ELISA).
- Results** : Out of the 95 microfilaremic Myanmar subjects, 91 cases were positive for the Og4C3 antigen assay and 72 cases were positive for the anti-

filarial IgG4 antibody test. The antigen test could not detect 4 microfilaremic individuals while the anti-filarial specific IgG4 antibody was not detectable in 23 infected cases. The sera from patients with *Brugia malayi*, *Onchocerca volvulus*, *Opisthorchis viverrini*, *Strongyloides stercoralis*, *Trichuris trichiura*, *Giardia lamblia* and hookworm showed negative results when tested with Og4C3 antigen.

Conclusions : The sensitivity of Og4C3 antigen assay (95.8%) for diagnosis of *Wuchereria bancrofti* was higher than the anti-filarial IgG4 antibody assay (75.8%).

Key words : *Wuchereria bancrofti*, Diagnosis, Specific circulating antigen, Anti-filarial IgG4 antibody.

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การตรวจแอนติเจนที่จำเพาะต่อพยาธิ *Wuchereria bancrofti* เพื่อการวินิจฉัยโรคเท้าช้าง.
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- ที่มาของปัญหา : การตรวจหาพยาธิโรคเท้าช้าง *Wuchereria bancrofti* โดยใช้กล้องจุลทรรศน์ ตรวจหาตัวอ่อนไมโครฟิลาเรีย จากเลือดของผู้ป่วยที่เจาะในเวลากลางวัน ต้องอาศัยเวลาและเป็นวิธีที่มีความไวในการตรวจพบต่ำ ดังนั้นการใช้วิธีวินิจฉัยที่ดีกว่าจึงจำเป็นในการประเมินความชุกของโรค และประเมินผลการควบคุมโรค
- วัตถุประสงค์ : เพื่อประเมินความไวของวิธีการตรวจหา *Wuchereria bancrofti* - specific Og4C3 antigen ในการตรวจวินิจฉัยโรคเท้าช้าง
- สถานที่ทำการศึกษา : อ.แม่สอด จ.ตาก
- รูปแบบการวิจัย : การศึกษาแบบ Cross-sectional
- ผู้เข้าร่วมในการศึกษา : ผู้ป่วยชาวพม่าที่ตรวจพบตัวอ่อนไมโครฟิลาเรียของพยาธิ *Wuchereria bancrofti* ในกระแสเลือด (Mf+) จำนวน 95 ราย เป็นผู้ชาย 22 ราย และ ผู้หญิง 73 ราย
- วิธีการทำการศึกษา : ผู้เข้าร่วมแต่ละรายจะได้รับการตรวจตัวอ่อนไมโครฟิลาเรีย ของเชื้อ *Wuchereria bancrofti* จากเลือดโดยใช้กล้องจุลทรรศน์ ส่วนของซีรัมจะนำมาตรวจ Og4C3 antigen และ anti-filarial IgG4 antibody โดยวิธี ELISA
- ผลการศึกษา : จากการศึกษาวงศ์พม่าที่ตรวจพบไมโครฟิลาเรีย จำนวน 95 ราย พบว่าวิธีการตรวจ Og4C3 antigen ให้ผลบวกจำนวน 91 ราย และผลลบจำนวน 4 ราย ส่วนวิธีการตรวจ anti-filarial IgG4 antibody ให้ผลบวกจำนวน 72 ราย และให้ผลลบจำนวน 23 ราย ผู้ที่มีพยาธิชนิด *Brugia malayi*, *Onchocerca volvulus*, *Opisthorchis viverrini*, *Strongyloides stercoralis*, *Trichuris trichiura*, *Giardia lamblia* และ hookworm ให้ผลลบต่อการตรวจ Og4C3 antigen
- สรุป : การตรวจหา Og4C3 antigen พบว่า มีความไวในการวินิจฉัยโรคเท้าช้างที่เกิดจาก *Wuchereria bancrofti* 95.8% ส่วนวิธี anti-filarial IgG4 antibody มีความไวในการวินิจฉัย โรค 75.8%

Despite recent advances in vector control and chemotherapy, lymphatic filariasis, caused by nematode parasites, mainly *Wuchereria bancrofti* and *Brugia malayi* is still a major public health problem in the tropics and subtropics. The disease seriously affects socio-economic status in many areas of the world. The clinical manifestations include adenolymphangitis, lymphedema, hydrocele, elephantiasis, and tropical pulmonary eosinophilia. However, the majority of patients in the endemic areas have asymptomatic microfilaremia, with the estimated 120 million infected persons,⁽¹⁾ there are 1.1 billion persons at risk of acquiring infection.⁽²⁾ In Thai people, lymphatic filariasis affects 3.7 per 100,000 population.^(3,4)

The infection begins when the third infective-stage larvae enter the skin from the labium of mosquito vectors during biting. They then, migrate through the blood circulation and develop into the fourth-stage larvae and adult worms. The adult worms live in the lymphatic system. After mating, the female will produce microfilariae in the blood circulation. The microfilariae will be taken up by the mosquito vectors during a blood meal and develop into the infective-stage larvae to complete the life cycle.

Recently, there has been an influx of Myanmar citizens seeking work in Thailand. These people bring in not only their cheap labour, but also many infectious diseases including bancroftian filariasis. The prevalence of bancroftian filariasis among them ranges from 2% to 5%.⁽³⁾ Furthermore, *Culex quinquefasciatus*, the main mosquito vector, is also prevalent in Thailand. *C. quinquefasciatus* has

not been reported to transmit lymphatic filariasis in Thailand. However, in the laboratory, *W. bancrofti* can develop into the infective stage in *quinquefasciatus*.⁽⁵⁾ Therefore, Thai people are at risk to acquire the infection.

The objective of this study was to evaluate the *W. bancrofti* -specific Og4C3 antigen assay for diagnosis of bancroftian filariasis. Anti-filarial specific IgG4 antibody assays were also evaluated and compared to the antigen assay. This study involved the microfilaremic Myanmar people working in Mae Sot District, Tak Province of Thailand.

The routine method for diagnosis of lymphatic filariasis is detection of microfilariae in peripheral nocturnal blood. This method is tedious, time-consuming and it is difficult to differentiate one filarial species from another.⁽⁶⁾ Furthermore, conventional parasitological procedures fail to identify amicrofilaremic infections or individuals with very low microfilaria levels.⁽⁷⁾ The Og4C3 monoclonal antibody has been developed to diagnose microfilaremic individuals and it has high specificity and sensitivity.^(8,9) The sensitivity of this antigen assay is the same whether serum samples are collected during the day or at night.^(10,11)

Anti-filarial IgG4 antibody was shown to enhance the specificity of immunodiagnostic assays for lymphatic filariasis.⁽¹²⁻¹⁵⁾ The filarial Og4C3 antigen and anti-filarial IgG4 antibody assays have never been applied for use in the endemic areas of Thailand. We report here the results of *W. bancrofti* -specific circulating antigen and anti-filarial specific IgG4 assays for detection of *W. bancrofti* infection in microfilaremic individuals.

Materials and Methods

Study population

The study population consisted of 95 Myanmar migrants who were microfilaremic. These people were working in Mae Sot District of Tak Province. Those Myanmar migrants harboured nocturnal periodic type *W. bancrofti*. They were recruited during the survey of bancroftian filariasis in Myanmar worker migrants at Mae Sot District, Tak Province during 1996-1997.^(4, 16)

Serum samples for ELISA

Two milliliters of venous blood were obtained from each person with a sterile technique. The sera were separated from the blood and stored at -20°C until used. The negative control sera were from non-infected healthy people living in non-endemic areas. Sera from patients infected with other parasites, including *Brugia malayi*, *Onchocerca volvulus*, *Opisthorchis viverrini*, *Strongyloides stercoralis*, *Trichuris trichiura*, *Giardia lamblia* and hookworm were also tested.

The assay for *W. bancrofti* -specific Og4C3 circulating antigen

The ELISA for detecting and quantifying *W. bancrofti* -specific Og4C3 antigen was performed as described (Trop-Ag *W. bancrofti*, JCU Tropical Bio Technology Pty Ltd, Townsville, Queensland, Australia). Briefly, polystyrene 96-well microtiter plates coated with Og4C3 monoclonal antibody and blocked with high nitrogen casein were used for the assay. One hundred microliters of each serum sample were boiled with 300 µl of sample diluent for 5 minutes. The supernatant was recovered after centrifuging.

Each well was given 50 µl of supernatant fluid and kept in a humid container for 1.5 hours at room temperature or overnight at 4°C. After washing with PBS/T (PBS, 0.05 % Tween 20), 50 µl of diluted anti-*Onchocerca* antibody were added to each well and kept for 1 hour at room temperature. After another wash, 50 µl of diluted sheep anti-rabbit immunoglobulin-horseradish peroxidase (HRP) conjugate was added to each well. The sample, hyperimmune rabbit antibody and conjugate were each diluted with blocking solution (PBS/T, 0.5% high nitrogen casein). Plates were kept for one hour at room temperature. After washing, 100 µl of ABTS substrates were added to each well. The plates were kept for 30 min at room temperature. The reaction was stopped with 50 µl of 2M H₂SO₄. The absorbance was read spectrophotometrically at 405 nm. The positive samples had antigen titers more than 100 units/ml.

Anti-filarial specific IgG4 antibody test

One hundred microliters of adult *B. malayi* extract in carbonate buffer (1 µg/ml) were coated onto each well of microtiter plates and incubated overnight at 4°C. After washing with PBS/T, each well was blocked with 100 µl of 2% nonfat dried milk (Carnation) in PBS/T. One hundred microliters of each serum sample were added to each well at 1:50 and 1:100 dilution. The plates were incubated for 1 hour at 37°C. After 5 washes, anti-human IgG4-horseradish peroxidase (HRP) conjugate (1:2,000) was added to each well and kept for 30 min at room temperature. After another 10 washes, 100 µl of a substrate mixture (O-phenylenediamine) was added to each well and kept at room temperature for 30 min. The

Table 1. Comparison of *W. bancrofti*-specific Og4C3 antigenemia and anti-filarial specific IgG4 antibody from microfilaremic patients.

Assay		Anti-filarial specific IgG4 antibody		
		Positive Number (%)	Negative Number (%)	Total Number (%)
Og4C3 antigen	Positive	71(74.7)	20(21.1)	91(95.8)
	Number (%)			
	Negative	1(1.1)	3(3.1)	4(4.2)
	Number (%)			
Total		72(75.8)	23(24.2)	95(100)
Number (%)				

The *W. bancrofti*-specific Og4C3 antigen and anti-filarial specific IgG4 antibody classified by sex

were female (Table 2). For the 72 cases who were positive for the anti-filarial specific IgG4 antibody, 21 cases were male and 51 cases were female.

Out of the 91 cases who were positive for the Og4C3 antigen test, 21 cases were male and 70 cases

Table 2. *W. bancrofti*-specific Og4C3 antigen and anti-filarial specific IgG4 antibody in microfilaremic patient classified by sex.

Sex	<i>W. bancrofti</i> -specific Og4C3 antigen		Anti-filarial specific IgG4 antibody	
	Positive Number (%)	Negative Number (%)	Positive Number (%)	Negative Number (%)
Male	21 (22.1)	1 (1.1)	21 (22.1)	1 (1.1)
Female	70 (73.7)	3 (3.1)	51 (53.7)	22 (23.1)
Total	91 (95.8)	4 (4.2)	72 (75.8)	23 (24.2)

Discussion

Generally, lymphatic filariasis affected males more than females.^(3, 17) However, there were more infected females in our study than males (Table 2). This was because most workers in the industries recruited for the study were females.^(4, 16) These Myanmar migrants carry, nocturnal periodic type, *W. bancrofti* with an infection rate of 4.9%.⁽¹⁶⁾ The sensitivity of the Og4C3 antigen assay in this study was 95.8% while previous studies from Faris and colleagues,⁽¹⁸⁾ More and Copeman,⁽⁸⁾ and Lammie and colleagues,⁽¹⁰⁾ showed sensitivities of 88%, 98.5% and 100%, respectively. It is possible that the microfilaremic persons, who were negative for the antigen test (Mf+ Ag-) had antigens below the detectable level due to the low level of microfilaremia and/or low adult worm burden.⁽¹⁹⁾ An alternative explanation is that host immune responses could clear the antigen to the undetectable level, or that the antigen forms the immune complex.⁽²⁰⁾ The anti-filarial specific IgG4 assay diagnosed lymphatic filariasis with a sensitivity of 94.5% in a study by Chanteau and colleague.⁽²¹⁾ We showed that the IgG4 assay could not detect 24.2% of the microfilaremic persons and could not detect 20 (22%) persons who had antigenemia (Table 1). While it is shown that IgG4 level decreases after treatment with diethylcarbamazine (DEC),⁽²²⁾ none of the microfilaremic Myanmar migrants in our study had received DEC. Therefore, it is possible that the antibody could not be detected due to an early infection as shown in another parasitic infection⁽²³⁾ or there is immune complex formation. The patients with lymphatic filariasis may also develop immunotolerance.⁽²⁰⁾

This Og4C3 monoclonal antibody was raised against *Onchocerca gibsoni* antigens found in both microfilarial and adult worms. The monoclonal antibody specifically detects circulating antigen in sera from patients with *W. bancrofti* but not those infected with other *Brugia*, *Oncocerca*, *Mansonella*, *Loa*, or common helminths such as *Ascaris*, *Trichuris*,^(8, 24) *Strongyloides*, *Opisthorchis*, *Giardia*, *Trichuris* and hookworm (data not shown). Though the sensitivity of the Og4C3 test (95.8%) was higher than the IgG4 assay (75.8%) (Table 2), the antigen could not be detected in a certain number of microfilaremic individuals which varied among the studies.^(8, 10, 18) Recently, it has been shown that there is no significant association between the rate or degree of microfilaria or antigen clearance and variables of age, sex, or absolute pretreatment microfilaremia levels.⁽²⁴⁾ Furthermore, the antigen levels do not clear rapidly in persons treated with either DEC or ivermectin. These data suggest that the antigen-detection assay may prove to be less useful than initially anticipated for purposes of community assessment after mass chemotherapy.⁽²⁴⁾ However, it should be noted that the antigen test could detect a portion of infected individuals who were amicrofilaremic.⁽⁸⁻¹⁰⁾

Together with strategies for treatment and control of the filarial parasites and mosquito vectors, highly sensitive and specific assays are required for evaluating the lymphatic filariasis control program. The highly sensitive antigen assay may facilitate the diagnosis of lymphatic filariasis. Nevertheless, the cost of these tests should be reasonable for use in poor endemic areas. Furthermore, public warning for the

risk and consequence of getting the infection, and health education for appropriate prevention methods, are the key elements for control of lymphatic filariasis as well as other infectious diseases.

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