ASIAN PACIFIC JOURNAL OF ALLERGY AND IMMUNOLOGY (2003) 21: 253-257

Comparative Assessment of an Og4C3 ELISA and an ICT Filariasis Test: A Study of Myanmar Migrants in Thailand

Surang Nuchprayoon[1,3], Chantima Porksakorn[1], Alisa Junpee[1], Vivornpun Sanprasert[1] and Yong Poovorawan[2]

SUMMARY **Detection of circulating filarial antigen has now emerged as an alternative method for the diagnosis of bancroftian filariasis. We compared two antigen detection assays, an Og4C3 ELISA and an ICT (immunochromatography) Filariasis test, for the diagnosis of *Wuchereria bancrofti* infections in migrant Myanmar workers in Tak province, Western Thailand. A total of 337 Myanmars participated in this study. The microfilarial rate was 3.3%. The Og4C3 ELISA could detect 19.1% of bancroftian filariasis while the ICT test detected 12.7%. Both antigen assays could detect all microfilaremics. The Og4C3 ELISA detected 14.8% of amicrofilaremics while the ICT test identified 8.1%. Those who were positive for the ICT test were also positive by the Og4C3 ELISA. Those Og4C3 positive cases, that were ICT negative (ICT-ve/Og4C3+ve) had statistically significant ($p < 0.05$, unpaired *t*-test) lower Og4C3 antigen levels (409.5 units, range 117-2,389) than those that were ICT positive (ICT+ve/Og4C3+ve) (5,252.0 units, range 130-28,062). Our results emphasize the problem of bancroftian filariasis in Myanmar migrants working in Thailand. Close monitoring and control of this disease in Myanmar migrants are of public health importance. Antigen detection systems are promising tools for the surveillance of bancroftian filariasis.**

Lymphatic filariasis, mainly caused by the parasitic filarial worms *Wuchereria bancrofti* and *Brugia malayi*, is still a major public health problem in the tropics. Over 90% of the disease burden is due to *W. bancrofti*.[1] The disease affects over 120 million people[2] and persists as the second leading cause of clinical debility and disability worldwide.[3] The World Health Assembly has set the goal to eliminate lymphatic filariasis as a public health problem.[2] To achieve this goal, the elimination program requires the collaboration of the public health systems in the affected countries. Furthermore, assessment and monitoring of the control programs by efficient surveillance procedures are needed to determine where control efforts should be initiated, how effective they are, and when they may be discontinued.[3]

Recently, it has been reported that Myanmar immigrants to Thailand carry *W. bancrofti* with a prevalence of 2%-8%,[4-7] while the prevalence in Thai people was only 1.62 cases/100,000.[8] Conventionally, diagnosis of bancroftian filariasis is based on the microscopic detection of microfilariae in blood samples taken at night.[6-7,9-10] Bancroftian filariasis is often underreported because people with a low level of microfilaremia or amicrofilaremia can be missed by the conventional microscopic examination.[11] Detection of circulating filarial antigen has now emerged as alternative pathway for the diagnosis of *W. bancrofti* infections.[7,10,12] Commercial kits utilizing specific monoclonal antibodies to

From the [1]Department of Parasitology, [2]Department of Pediatrics and [3]Chula Medical Research Center, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand
Correspondence: Surang Nuchprayoon

detect *W. bancrofti* antigens are currently available. An enzyme-linked immunosorbent assay (ELISA) based on Og4C3 monoclonal antibody[13] has been used to diagnose microfilaremic as well as amicrofilaremic individuals with high specificity and sensitivity.[9,13] Recently, a whole blood immunochromatographic (ICT) filariasis card test has been developed using the AD 12.1 monoclonal antibody,[14] with high specificity and sensitivity to *W. bancrofti* antigen.[15] The card test is a promising field-ready diagnostic tool due to its ease of use.[15]

In the present study, we evaluated the burden of bancroftian filariasis in Myanmar migrants in Thailand by comparing the ICT Filariasis test to the Og4C3 ELISA for antigen detection.

MATERIALS AND METHODS

Study population

The study subjects were Myanmar migrants recruited from two factories in Mae Sot District, Tak Province, Western Thailand. Verbal informed consent was obtained from each adult or child's parent or guardian in the presence of two witnesses. Individuals who were sick or had a history of receiving diethylcarbamazine (DEC) treatment were excluded from this study. Signs and symptoms of lymphatic filariasis were established through an interview and physical examination. A translator who spoke Thai and Myanmese helped with the communication. This study was approved by the Ethics Committee of the Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand.

Blood specimens and parasitological analysis

Two to five milliliters of venous blood were collected from 337 individuals by sterile technique and universal precaution between 8.00 p.m. and midnight. Thick-blood films were prepared in duplicate as described previously.[6-7,16-18] All of the microfilaria-postive specimens were *W. bancrofti.*

Due to difficulties in transportation, a total of 220 whole blood samples and 272 sera were available for the ICT Filariasis test and Og4C3 ELISA, respectively. Sera from non-infected healthy people living in non-endemic areas were used as negative controls.

Detection of *W. bancrofti* specific antigens

The Og4C3 circulating antigen detection test was done by a sandwich ELISA[13] according to the manufacturer's recommendations (TropBio Pty Ltd, Townsville, Australia).

The whole blood version of the ICT test kit (AMRAD Operations, New South Wales, Australia) was performed according to the manufacturer' s instruction for the qualitative detection of *W. bancrofti* antigen.

Data analysis

The data were collected and analyzed using Excel 6.0® software as well as the unpaired *t*-test and the Chi-square test with the level of significance set at $p < 0.05$.

RESULTS

Among the 337 Myanmar migrant workers, 58 (17.2%) were male and 279 (82.8%) were female (Table 1). The mean age of the study population was 22.3 ± 6.0 years (range 10-56 years). Clinical manifestations of lymphatic obstruction were observed in 13 individuals. Eight individuals had enlarged inguinal lymph nodes. Five individuals had enlarged scrotums. By using blood smear examination under a microscope, microfilariae were detected in 11 (3.3%) individuals, 2 of whom were male and 9 were female.

Table 1 Prevalence of bancroftian filariasis in Myanmar migrants classified by sex, and diagnostic methods

Test	No. examined (%)			No. positive (%)		
	Total	Male	Female	Total	Male	Female
Thick-blood film	337	58 (17.2)	279 (82.8)	11 (3.3)	2 (3.4)	9 (3.2)
ICT filariasis test	220	47 (21.4)	173 (78.6)	28 (12.7)	4 (8.5)	24 (13.9)
Og4C3 ELISA	272	47 (17.3)	225 (82.7)	53 (19.5)	6 (12.8)	47 (20.9)

The prevalence of filarial antigenemia was 12.7% (28 of 220) as assessed by the ICT Filariasis test, and 19.5% (53 of 272) as assessed by the Og4C3 ELISA (Table 1). The ICT Filariasis test identified 4 antigen-positive males and 24 females, resulting in prevalence rates of 8.5% and 13.9%, respectively. Out of the 53 Og4C3 antigenemic cases, there were 6 (12.8%) antigen-positive males and 47 (20.9%) females. There was no statistical significance in gender differences in the prevalence as assessed by thick-blood films, the ICT Filariasis test, and ELISA for Og4C3 antigen.

The data were further analyzed to compare the prevalence rates for bancroftian filariasis as assessed by thick-blood film, the ICT Filariasis test and Og4C3 ELISA (N = 220). Forty-two (19.1%) individuals were positive for at least one test, all of which were positive for Og4C3 antigen. Both antigen detection tests identified all microfilaremic cases. All the 28 ICT-positive cases were also positive with the Og4C3 ELISA. In amicrofilaremic individuals, the positive rate of the ICT Filariasis test for specific *W. bancrofti* circulating antigen was 8.1% (17 of 209) while the ELISA for Og4C3 antigen could detect 14.8% (31 of 209) of amicrofilaremics (data not shown).

The Og4C3 antigen levels for the ICT+ve/Og4C3+ve, ICT-ve/Og4C3+ve, and ICT-ve/Og4C3-ve groups were 5,252.0 (130-28,062, N = 28), 409.5 (117-2,389, N = 14), and 10.4 (10-61, N = 178) units, respectively (data not shown). Among the Og4C3 positive cases, those with ICT negative (ICT-ve/Og4C3+ve) had statistically significant lower Og4C3 antigen levels than the ICT positive (ICT+ve/Og4C3+ve) individuals ($p < 0.05$, unpaired *t*-test).

DISCUSSION

The antigenemic status of *W. bancrofti* infection among migrant Myanmar workers was assessed using the ICT Filariasis test and an ELISA for Og4C3 antigen detection. Previous baseline data on the prevalence of bancroftian filariasis among migrant Myanmar workers in Thailand were based on parasitological diagnosis using thick film bloodsmears.[5,8] Similar to previous studies which estimated that 2%-8% of these migrants carry *W. bancrofti* infection,[4-6] we found that the migrants had a microfilarial rate of 3.3%, while both antigen tests iden-tified 2-5 times more cases (Table 1).

It is estimated that hundreds of thousands of Myanmar migrants have settled in the urban cities of Thailand. The infected immigrants carry the nocturnal periodic form (urban type) of *W. bancrofti* which has *Culex quinquefasciatus* as the main vector species.[7,19] *Cx. quinquefasciatus* is abundant in the big cities of Thailand, and has the potential to transmit bancroftian filariasis[19] thus putting the Thai population at risk of acquiring this infection. Therefore there is a major concern that bancroftian filariasis may re-emerge as a major health problem for Thai citizens. It is necessary to set up well-planned strategies for the control of lymphatic filariasis while the Myanmar migrants are working in Thailand. Obviously, using improved diagnostic methods is essential to facilitate surveillance activities, and monitor and evaluate control efforts.

Both diagnostic kits used in this study detected antigens secreted from adult worms,[14,20-21] indicating active infection. As both antigen detection tests were capable of detecting *W. bancrofti* circulating antigens in microfilaremic as well as amicrofilaremic individuals, the use of thick-blood films would have underestimated the real prevalence by 2-5 folds in this population (Table 1). The fact that the prevalence would be higher when using antigen tests for screening instead of microscopic examination for microfilariae was reported previously.[7,10,15,22-26] Circulating antigens as markers for the diagnosis of bancroftian filariasis can detect individuals with low levels of or absent microfilaremia.[24,27] Furthermore, large numbers blood specimens can be obtained during daytime fast and easily. As part of the global elimination program, antigen testing would therefore be a useful rapid screening tool for determining the prevalence and distribution of *W. bancrofti*.[15,28]

As reported previously,[23,29-33] the ICT Filariasis test and Og4C3 ELISA could detect all microfilaremic cases. The Og4C3 antigen levels of our microfilaremic group were significantly higher than of the amicrofilaremic group ($p < 0.05$, unpaired *t*-test; data not shown).

We found that the ICT Filariasis test could detect less patients with active *W. bancrofti* infection than the Og4C3 ELISA (Table 1), as previously described.[26,32] This indicates that the Og4C3 ELISA is a more sensitive test. In support of this hypothesis, all ICT-positive (ICT +ve) individuals

were also positive for the Og4C3 antigen. Also those in the ICT-ve/Og4C3+ve group had statistically significant lower antigen levels than the ICT+ve/ Og4C3+ve group. The Og4C3 antigen is released from adult worms and is thought to correlate with the adult worm burden.[20] It is possible that the ICT-ve/Og4C3+ve group had a lower adult worm burden than the ICT+ve/Og4C3+ve group. Alternatively, the Og4C3 monoclonal antibody (mAb) may recognize more epitopes compared to the ICT's AD 12.1 mAb. Furthermore, the test protocol of the Og4C3 ELISA includes a boiling step to release antigen possibly trapped inside the immune complexes, which increases the chance of detection by the Og4C3 mAb.

While the global elimination program of lymphatic filariasis is ongoing, highly sensitive and specific diagnostic assays are necessary to monitor and control the program. This study demonstrated the value of antigen detection tests as a means of surveillance for bancroftian filariasis in a migrant Myanmar population. The Og4C3 ELISA has a higher performance in detecting amicrofilaremics than the ICT Filariasis test. However, the ICT Filariasis test may be more appropriate in remote areas because of its ease of use, as finger-prick blood is sufficient and results are obtained within 15 minutes.

ACKNOWLEDGMENTS

We are thankful for the support from the Thailand Research Fund (TRF) and the UNDP/World Bank/WHO Special Program for Research and Training in Tropical Diseases. CP and VS are Ph.D. students in the Royal Golden Jubilee (RGJ) Ph.D. Program supported by TRF. AJ is a graduate student supported by National Center for Genetic Engineering and Biotechnology (BIOTEC). YP is a Senior Research Scholar of the Thailand Research Fund. We are thankful to Dr. Saravudh Suwanadabba (Ministry of Public Health) for his helpful advice, to Ms. Kobkarn Karnjanopas for co-ordination in the field, to Ms. Songpun Sangprakan, and Ms. Chantharat Krainara for technical assistance, and to Ms. Jiraporn Songtrus and all the health personnel from Vector-borne Diseases Center 18 and the Filariasis Division, CDC Department, Ministry of Public Health; and the staffs at the Department of Parasitology, Faculty of Medicine, Chulalongkorn University, for their technical assistance in the field and laboratory work.

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