PARASITOLOGICAL AND SEROLOGICAL INVESTIGATION INTO LYMPHATIC FILARIASIS AMONG IMMIGRANTS AT SEMENYIH DETENTION CENTRE, SELANGOR, WEST MALAYSIA

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ABSTRACT: Parasitological and serological investigations for lymphatic filariasis were performed on 450 immigrants detained at the Immigration Centre at Semenyih, Selangor, West Malaysia. The country of origin of these immigrants were Indonesia, The Philippines, Myanmar, Bangladesh, India and Pakistan. *Brugia malayi* adult worm homogenate (BmAH) antigen was used for the detection of antifilarial IgG. A monoclonal antibody-based ELISA (MAb.XC3-ELISA) specific for filarial circulating antigens and non-phosphorylcholine reactive was used to detect antigenemia in these immigrants. Parasitologically 67 (14.89 %) were positive for *W. bancrofti* and 54 (12.0%) for *Brugia malayi*. Serologically 63 % had antifilarial IgG titre to the BmAH antigen. While Bancroftian filariasis is now unknown in Peninsular Malaysia, the potential of it to be reintroduced into Peninsular Malaysia by the immigrant population is discussed. *(JUMMEC 2000; 1: 41-44)*

KEYWORDS: Lymphatic filariasis, immigrants, antifilarial IgG, antigenemia

Introduction

During the last few years before the economic slow down, there has been an influx of immigrants into Malaysia. Due to better economic prospects thousands of immigrants have entered Malaysia in search for a better life. The immigrants are employed in various economic sectors such as domestic maids, agriculture, building and construction. The government of Malaysia has tightened the immigration policy. Those seeking employment must obtain work permit in addition to proper and valid travelling documents. Failture to produce these documents lead to deportation. Pending deportation they will be detained at Imigration Dentention Center. There is need to ensure that Malaysia is not burdened by the need for care of immigrant with health problems as well as the increased risk posed to the local population from exposure to communicable disease.

Lymphatic filariasis is endemic in Asia. The infection persist as a major cause of clinical morbidity and a significant impediment to socioeconomic development. Its prevalence is increasing world wide, largely because of rapid unplaned urbanization in many endemic areas. It is estimated that at least 120 million people are infected. The sub-periodic form of *Brugia malayi* occurs mostly in the swamp forest areas of Malaysia and has a wide animal reservoir which includes monkeys. The periodic form is endemic mainly in the coastal rice field regions of Malaysia (3, 4). In Malaysia *W. bancrofti*, especially in the cities have been eliminated. However their vectors especially those responsible for the trasmission of *W. bancrofti* breeds in abundance in the cities. With the influx of immigrants and in relation to their occupational nature, the whole facet of lymphatic filariasis in Malaysia may change.

We report in this paper our parasitological and serological findings on the prevalence of lymphatic filariasis among immigrants detained at the Semenyih Immigration center. If the trend described in this paper persist, one would be able to predict the forthcoming

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health problems pose to this cpuntry by the migrant population. This will help the design and planning of health services for migrants to be undertaken on a more rational basis.

Materials and methods

Subjects

The sample population consisted of 450 male immigrants and their age range from 12 years to 60 years majority being in the 21 to 33 years age group. The country of origin of these immigrants are Indonesia, The Phillipines, Bangladesh, Myanmar, India and Pakistan.

Sera

Blood samples were taken by veinpuncture between 8 to 12 P.M. Sera were seperated from blood samples and stored at –20° C until used. The presence of microfilaremia was detected by microscopic examination of stained thick smears of blood (60 µl) obtained at night. Controls were residents of Perak Tengah, an endemic area for *Brugia malayi*. These individuals were with symptoms of chronic disease and of acute filariasis (adenitis, lymphangitis, or pulmonary eosinophilia), and also were microfilaremic. In addition normal individuals from non-endemic area were also included as negative controls.

Antigens

Brugia malayi adult homogenate antigen (B.m.AdH) was used to titrate the antifilarial IgG antibodies. B.m.AdH was prepared from 50 adult worms recovered from infected gerbils by peritoneal lavage. Worms were homogenized in sterile normal saline and were sonicated using Dynatech Sonic Dismembrator at 6 kilocycles MHZ for 3 times on a 3 minutes off and 3 minutes on pattern in ice. To ensure proper fragmentation, a drop of suspension was examined under light microscope. Crude mixture was kept at 4° C for overnight and again centrifuge at 2000 rpm for 10 minutes. The supernatant was removed and centrifuged at 1300 rpm for 20 minutes at 4° C. Finally the supernatant was lyophilized, aliquotted and stored at –2° C until use. Protein concentration was determined by Lowry's method (7).

Enzyme immunoassay (EIA)

A monoclonal antibody designated as MAbXC3 was used in EIA for the detection of antigenemia in these subjects. MAbXC3 is a non-phosphorylcholine reactive mouse IgG 1 (1-2; 5-6). Briefly, serum specimens were treated to release antigens from immune complexes and to remove interfering proteins prior to performing the monoclonal-based EIA (MAbXC3-EIA). Serum specimens were diluted with an equal volume of 0.1 M disodium EDTA (pH 7.5), heated 5 min on a boiling water bath and microcentrifuged at 16,000 x g for 7 min before testing in the assay. Preliminary studies demonstrated that the procedure provides more than 90 % recovery of antigen activity. The optimal dilutions of B.m.AdH antigens and the monoclonal antibody (MAbXC3) were determined by chequer board titration. Polyvinyl microtiter plates (Dynatech Lab., Alexandria, VA, USA) were sensitized by overnight incubation at 37° C with 100 µl/well MAbXC3 (20 µg/ml in 0.1 M Na HCO3, pH 8.0). Serum specimens that have been serially diluted were added to the sensitized microplates in triplicates (50 µl/well). After two hour, the plates were washed with PBS with 0.05% Tween 80 (Sigma Chem. Co.) and 100 µl/well MAb.XC3 was again added. The plates were incubated at 37 °C for 1 hr. After washing, 50 µl of an appropriate dilution of peroxidase conjugated goat antisera against mouse IgG1 (KPL, Gaithesburg, MD) was added. After washing the plates were developed with 2-azino-di-(3-ethylbenzthiazoline sulfonic acid) substrate for 30 min. The reaction was stopped with 50 µl 4 M H_2SO_4. Optical density was read *vs* a PBS blank at 405 nm with an ELISA reader (Titertek Multiscan, Linbro, Hamden, CT, USA). Since 3 of 20 non-endemic normal sera gave positive reaction at serum dilution of 1:150 when sceened for antigen, we considered 1:300 titre as positive for filarial antigen and the sera were screened at 1:300 dilution.

Direct EIA was performed to detect antifilarial IgG antibodies to B.m.AdH. Binding of antifilrial IgG in the serum samples from these immigrants was detected using peroxidase labelled goat anti-human IgG (KPL, Gaithesburg, VA, USA). Since 3 of 20 non-endemic normal sera gave a positive reaction at serum dilution of 1:80 when screened for antifilarial IgG, we considered 1: 160 as positive reaction for filarial antigen and the sera were sceened at 1:160 dilution.

Results and Discussion

Parasitologically, microfilaremia with *W. bancrofti* were detected in 67 (14.89 %) of the 450 immigrants (Table 1). Microfilaremia with *B. malayi* were detected in 54 (12.0%). Microfilaremic cases were detected the highest among the Indonesian immigrants (77 cases) followed by Bangladeshi (32 cases) and their total numbers were 180 and 120 respectively which were also higher than others.

The antifilarial IgG titers of these immigrants to B.m. AdH are shown in Table 2. A panel of 20 well-characterized sera of local Malaysians from Perak Tengah an endemic area and normal individuals from non-endemic area were included as positive and negative controls respectively. The highest titer (5120) was seen among the Indonesians (n=15).

Table 3 shows the profile of filarial circulating antigen titer. 155 (34.44 %) were antigenemic. Antigenemic cases were high among the Indonesians and the Bangladeshi. The highest titer (9600) were seen among

Table 1. Microfilaremic cases detected among immigrants according to country of origin.

Country of Origin	No. tested	No. positive (%)	No. of microfilaremic cases (%) W. bancrofti	B. malayi
Indonesia	180	77 (17.1)	42 (9.3)	35 (7.8)
Philippines	45	3 (0.7)	2 (0.4)	1 (0.2)
Myanmar	60	5 (1.1)	3 (0.7)	2 (0.4)
India	30	2 (0.4)	1 (0.2)	1 (0.2)
Bangladesh	120	32 (7.1)	18 (4.0)	14 (3.1)
Pakistan	15	2 (0.4)	1 (0.2)	1 (0.2)
Total	450	121 (26.9)	67 (14.9)	54 (12.0)

Table 2. Detection of antifilarial IgG employing BmAdHom antigen in direct EIA.

Country of origin	No. tested	No. positive (%)	Reciprocal of antifilarial IgG 160	320	640	1280	2560	5120
Indonesia	180	18 (40.0)	15	21	36	21	33	54
Philippines	45	27 (6.0)	-	-	3	9	3	12
Myanmar	60	37 (8.22)	-	1	2	10	9	15
India	30	5 (1.11)	-	-	-	-	-	5
Bangladesh	120	81 (19.33)	2	4	6	15	21	33
Pakistan	15	1 (0.22)	-	-	-	-	-	1
Total	450	331 (74.89)	17	26	47	55	66	120

Table 3. Detection of filarial circulating antigen employing MabXC3-EIA.

Country of Origin	No. tested	No. positive (%)	Reciprocal antigen titre 300	600	1200	2400	4800	9600
Indonesia	180	81 (18.0)	33	24	9	6	6	3
Phillipines	45	12 (2.7)	6	3	2	1	-	-
Myanmar	60	15 (3.3)	7	6	1	1	-	-
India	30	3 (0.7)	2	1	-	-	-	-
Bangladesh	120	42 (9.3)	15	9	7	6	3	2
Pakistan	15	2 (0.4)	1	1	-	-	-	-
Total	450	155 (34.4)	64	44	19	14	9	5

3 Indonesians and 2 Bangladeshi. All these immigrants come from countries which are endemic for lymphatic filariasis. In an endemic population microfilaremia and clinical manifestations may be absent, such as during early infections. Therefore the diagnosis of lymphatic filariais is then extremely difficult. Nevertherless most of them may exhibit positive skin tests should they are subjected to challenge with antigenic extracts of human and animal filariids since most of them invariably contain antifilarial IgG. Similarly, the antifilarial antibodies can be detected by a variety of serological assays (8-10). However, these reactions persist for many years even when control programs are effective in interrupting transmission (11- 12). Sharing of antigens between different filarial species and between filariae and other nematodes further limits the usefulness of antibody assays for filariasis. (5, 6).

Diagnostic assay based on the detection of parasite antigen in biological specimens with highly specific monoclonal antibodies would alleviate some of these shortcomings (10- 12). The MAbXC3-EIA that has been developed and used in this study should serve the purpose. To be applicable in the field setting of rural Malaysia where laboratory facilities are inadequate, the

assay protocol can be modified to make it more simpler to perform. With these considerations in mind, a dipstick assay in which the MabXC3 (which is specific to serum filarial antigen) retained on nitrocellulose membrane would be a suitable format. Patients serum can be dotted directly to such portable dipstick which has potential for field application and in this case immigrants entering Malaysia.

Finally the findings from this study appear to show that foreign workers may pose a sizeable amount of health problems especially with regard to *W. bancrofti* transmission. There is potential of Bancroftian filariasisis may eventually be reintroduced into Peninsular Malaysia through these immigrants. Screening can be conducted on every immigrant entering Peninsular Malaysia and screening for lymphatic filariasis should be included together with other communicable diseases. If these are not address quickly it may endanger the health of this country, while we readily acknowledge their contribution towards national development.

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