Malaysian Journal of Medical Sciences, Vol. 8, No. 2, July 2001 (19-24)

ORIGINAL ARTICLE

EPIDEMIOLOGICAL SCREENING OF LYMPHATIC FILARIASIS AMONG IMMIGRANTS USING DIPSTICK COLLOIDAL DYE IMMUNOASSAY

Wan Omar, A, Sulaiman, O[*], Yusof, S[*], Ismail, G[*], Fatmah, M.S[*]., Rahmah, N[**] and Khairul, A.A[***].

Department of Clinical Laboratory Sciences, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

[*]Department of Parasitology and Medical Entomology, Faculty of Medicine, Universiti Kebangsaan Malaysia, Jalan Raja Muda Abdul Aziz, 50300 Kuala Lumpur, Malaysia

[**]Deparment of Microbiology and Parasitology, School of Medical Sciences, School of Medical Sciences, Universiti Sains Malaysia 16150 Kubang Kerian, Kelantan, Malaysia

[***]Department of Parasitology, Faculty of Medicine, Universiti Malaya, 50603 Kuala Lumpur, Malaysia

We have recently reported that a dipstick colloidal dye immunoassay (DIA) that detect parasite antigens in human serum is sensitive and specific for the diagnosis of active infection of lymphatic filariasis. Rabbit polyclonal antibodies (RbBmCAg) labelled with a commercial dye, palanil navy blue was used to detect filarial antigenemia among Indonesian and Bangladeshi immigrant workers (N= 630) at oil palm estates at Hulu Trengganu District, Peninsular Malaysia. Microfilaremia with *Brugia malayi* were detected in 51 (8.10 %) individuals, of which 42 (6.67 %) were among the Indonesians and 9 (1.98 %) among the Bangladeshis. Microfilaremia with *Wuchereria bancrofti* were detected in 33 (5.24 %) individuals of which 15 (2.38 %) were among the Indonesians and 18 (2.86 %) among the Bangladeshis workers. The DIA detected 96 (15.24 %) antigenemic cases which comprise of all the microfilaremic cases and 15 (2.38 %) amicrofilaremic cases. The amicrofilaremic cases with filarial antigenemia consisted of 9 (1. 43 %) Indonesians and 6 (0.95%) Bangladeshis. We have used 6 ul of the RbBmCAg and diluted (1:10) patients' sera per dipstick which make the DIA reagent conservative. The DIA is a rapid test and can be read in approximate 2 hours.. Additionally, coloured dots developed in the DIA can be qualitatively assessed visually for intensity. The DIA does not require sophisticated equipment or radioactivity, and therefore suitable for field application.

Key words : Dipstick , Colloidal Dye, Immunoassay, Filariasis

Submitted-12.6.2000, Revised Article-4.1.2001, Date Accepted-15.5.2001

Introduction

With the increasing ease of travel and the passage of peoples between countries there is an increase risk posed to the local population from transmission of vector-borne diseases. Due to better economic prospect immigrants have entered Malaysia in search for a better life. The immigrants are employed in various economic sectors such as domestic maids, estates and construction sectors.

Lymphatic filariasis caused by *Brugia malayi* and *Wuchereria bancrofti* is a major public health problem in many tropical countries. A minimum of 120 million people in 73 endemic countries world-

wide are estimated to be infected (1, 2). It is currently estimated that some 512 million people are at risk of infection in sub-saharan Africa, with about 28 million people already infected (3, 4). The species *Brugia malayi* however is predominantly found in Southeast Asian countries. Most programmes for control of lymphatic filariasis include mosquito control measures, diagnostic screening and drug therapy for suppression of microfilaremia. Much effort has recently been devoted to the development of tests that allow the indirect diagnosis of active infections based on the detection of worm antigens in biological specimens such as sera and urine. Several methods to detect filarial antigens in sera have been described (5- 9). Antigen detection assays have several advantages over other diagnostic methods (10-13). They can identify individuals with pre-patent or occult infections which are undetected by conventional parasitologic tests, give a more accurate indication of active infection than traditional serodiagnostic tests, and they provide an indirect measurement of parasite burdens that can be used to monitor the efficacy of control programs or therapeutic interventions. The enzyme linked immunosorbent assay (ELISA) which is widely in use has limitations of its utility under field conditions. In such situations, a simple, inexpensive colorimetric dipstick assay using robust reagents and no instrumentation could have many diagnostic applications (14-15).

Our purpose here is to screen immigrants working in the agricultural estate sector for filarial infections. Serological investigation was accomplised using a dipstick colloidal dye immunoassay (DIA) which we have recently developed. The DIA detect parasite antigens present in sera from humans infected with *Brugia* spp and *Wuchereria bancrofti* but absent from sera infected with nonfilarial helminths (15). We now report screening of lymphatic filariasis among estate immigrant workers using this simple, rapid and inexpensive immunological test.

Materials and Methods

Antigen for raising polyclonal antibodies was from *Brugia malayi* adult worms collected from the peritoneal cavities of infected jirds. In brief the adult worms were washed in PBS, homogenized and sonicated. The extract was obtained by centrifuging at 20 000 x g at 4 °C for 20 min and the protein content was determined by u.v absorbence at 280 and 290 nm and adjusted to 15 mg/ml. The polyclonal antibodies against *B. malayi* circulating antigens were raised in rabbits (RbBmCAg) by immunizing with a mixture of 0.75 ml of the antigen in 0.75 ml Freund's complete adjuvant subcutaneously at weekly interval for three such doses. The rabbits were bled for sera 10 days after the last dose and sera were seperated by centrifugation and stored at –20 °C. Our previous studies indicated that the polyclonal RbBmCAg bind specifically to circulating antigens of *B. malayi* and *W. bancrofti* but not to circulating antigens of

Table 1. Prevalence of B. malayi *and* W. bancrofti *microfilaremia among the immigrants by finger prick blood smear examination*

Nationality	No. tested	No. positive	Microfilaremia	
			B. malayi	W. bancrofti
Indonesian	390 (61.90)	57 (9.05)	42 (6.67)	15 (2.38)
Bangladeshi	240 (38.10)	27 (4.48)	9 (1.98)	18 (2.86)
TOTAL	630 (100.0)	84 (13.33)	51 (8.10)	33 (5.24)

* Percentages are in parantheses

nonfilarial helminths (15).

A commercial palanil navy blue colloidal dye (Hoerchst, F.R.G) was used. Dye particle suspension was prepared using a washing/centrifugation procedure similar to that previously described by Gribnau *et. al* in 1983 (16). Briefly a 5% suspension of the colloidal dye in water (w/v) was washed four times by centrifugation at 20,000 x g for 30 min and resuspended in the same volume of water. A final low speed centrifugation (125 x g, 30 min) removed aggregated colloidal particles. The supernatant was decanted and 0.01 % thiomersal was added as preservative to produce the stock colloidal dye particle suspension.

The optimum wavelength for detection and quantitation of the dye was determined using visible spectrophotometric scan (Pye Unicam SP8-500 UV/ VIS, Philips). A small volume of the dye particle suspension was solubilized in ethanol for spectral scan. Optimum dye/Ab suspension which gave strongly coloured positive dot without an increased background colour was at the antibody concentration of 10 ug/ml.

The RbBmCAg was linked to the colloidal particle dye using a modification of a previously described technique by Gribnau *et al* (1983) (16). The dye/Ab absorbing condition was at 10 mM PO4 buffer with 10 mM NaCl at pH 7.2. The solution was then spiked with v/5 volume of a 30% bovine serum albumin (BSA) solution in 5 mM NaCl (pH 7.4) and incubated for another 1 hr to stabilize the dye particle surfaces. The dye/Ab reagents were centrifuged at 12,000 x g and the pellet was resuspended in a 33.3 mM phosphate, 0.125 M NaCl solution (pH7.4) containing 5 % BSA and 0.01 % thiomersal and stored at –20°C.

All the immigrant workers (N= 630) studied in this survey were employed by the oil palm estates of RISDA at Hulu Trengganu, Peninsular Malaysia They were from Indonesia (n= 390) and Bangladesh (n=240). These immigrant workers were relatively not so young as the mean age was 30 years and the majority were aged between 21 – 30 years. Most of the Indonesian workers were from Jawa Timur and Jambi, Sumatra, while majority of the Bangladeshis were from two neighbouring administrative districts of Dhaka and Chitagong.

Venous blood was collected form these workers between the hours of 8:30 p.m and midnight. Microfilaremia was determined by using 60 ul of thick and thin blood smears. Both films were dried overnight and stained with giemsa to examine for the presence of microfilariae. Serum was seperated from venous blood and stored at –20°C and later tested by the dipstick colloidal dye immunoassay. All subjects were personally interviewed and examined by one or more of the authors. A clinical diagnosis of filariasis was made in subjects with lymphoedema of the extremities with or without a history of recent lymphadenitis with retrograde lymphangitis and fever. Patients who had received diethylcarbamazine treatment in the past 12 months were excluded from the study. The controls included in the DIA were well characterized *B. malayi* infected Malaysian patients as positive controls (n=10), and normal healthy individuals as

Table 2 *Prevalence of filarial antigenemia among* B. malayi *(B..m MF),* W. bancrofti *(W.bMF) and amicrofilaremic (AMF) individuals by dipstick colloidal dye immunoassay*

Nationality	No. tested	No. positive	Antigenemia		
			B. m MF	W. b MF	AMF
Indonesian	390 (61.90)	66 (10.48)	42 (6.67)	15 (2.38)	9 (1.43)
Bangladeshi	240 (38.10)	33 (5.24)	9 (1.43)	18 (2.86)	6 (0.95)
TOTAL	630 (100.0)	96 (15.24)	51 (8.10)	33 (5.24)	15 (2.38)

* Percentages are in parantheses

negative controls (n=10). The positive controls were well-characterized individuals residing in a *B. malayi* endemic area of Perak Tengah, West Malaysia. Sera from Indonesian immigrants (n=6) infected with *W. bancrofti* were also included as another positive control. These sera were obtained from immigrants detained at the Semenyih Immigration Detention Center, Kajang, Malaysia. They were microfilaremic individuals with acute clinical signs or symptoms of inguinal lymphadenitis, lymphangitis and leg lymphoedema. Negative control sera were from well-characterized healthy adults residing in Kuala Lumpur City who had no history of residence in any filariasis-endemic area of Malaysia.

Dipsticks 1 x 8 cm in size were prepared by adhering nitrocellulose membrane (NC) to acetate sheets using double sided cellotape. The NC on each dipstick was divided into 3 segments, viz, segments for positive control, negative control and the patient serum to be tested for antigenemia. Well characterized sera from *B. malayi* infected patients as positive control and sera from normal unifected individual as negative controls were consistently incorporated into every dipstick for quality control. The capture layer was 6 ul of 10 ug/ml of RbBmCAg dotted on the NC segments of the dipstick. The antisera dots were allowed to dry on the NC strips. Our observations on several preliminary trials indicated that these strips could be used immediately, stored dry at room temperature or 4°C without compromising on the results of the dipstick test. The remaining protein binding sites on the NC surface were blocked with Tween 20 (Sigma) as 1 % solution in PBS (pH 7.4) for 30 min. The dipsticks were incubated at 37°C with the patients sera for 1 hr and then washed for 15 sec under gently running tap water. Dots of dye/Ab detecting reagent were placed on the dipsticks and incubated for 1 hr at 37°C. Dipsticks were then briefly rinsed in tap water, and blue colour dots for positive antigenemia were observed.

Results

Results of typical DIA tests for filarial antigenemia in the three groups of patients are presented in Fig 1. Positive reactions (two blue dots) were observed on the NC strips (previously dotted with RbBmCAg) for microfilaremic patient (A) and amicrofilaremic patient (B). Only one blue dot at the segment for positive control is seen in dipstick C which represent typical DIA result for normal uninfected individual that was negative for filarial antigenemia. Dots were blue with distinct border within the window of the mask. Negative results were obtained with sera of normal non-endemic controls. They were usually completely white, but were sometimes indistinct or lacked a distinct border.

Out of a total 630 blood samples of these immigrant workers tested, 84 (13.33 %) were found to have either periodic *B. malayi* or *W. bancrofti* microfilaremia (Table 1). Among the 390 Indonesian workers tested, 57 (9.05%) were microfilaremics, 42 (6.67%) of them were with *B. malayi* microfilaremia and 15 (2.38%) were with *W. bancrofti* microfilaremia. Twenty seven (4.48%) Bangladeshi immigrants were microfilaremic whereby 9 (1.98%) were with *B. malayi* and 18 (2.86 %) were with *W. bancrofti*.

Table 2 shows prevalence of antigenemia among the microfilaremic and amicrofilaremic individuals detected by the DIA. Fifteen (2.38%) antigenemic cases were detected among the amicrofilaremic individuals, 9 (1.43 %) and 6 (0.95 %) among the Indonesians and Bangladeshis respectively. Two Indonesians and one Bangladeshis had inguinal lymphadenitis and pyrexia. Other lymph nodes were not affected. None of these immigrants had lymphoedema or lymphangitis of the legs. In lymphatic filariasis the presence of enlarged lymph nodes is not a consistent sign of early infection. The presence of enlarged nodes does not indicate lymphatic filariasis; the absence certainly does not exclude it. We strongly believe that the enlarged nodes in these three microfilaremic cases were not due to other causes such as scabies or septic sore of the legs. These two Indonesian cases had microfilarial density of 432 m/ml bloodand 363 mf/ml blood. The one Bangladeshi case had microfilarial density of 420 mf/ml blood. Similarly, two amicrofilaremic cases (one Indonesian and one Bangladeshi) but positive by DIA had filarial related enlarged inguinal lymph nodes.

Discussion

The routinely in use microplate ELISA although specific and sensitive requires the use of spectrophotometer which is an expensive and sophisticated apparatus that is not available or useable in many underdeveloped areas of the world. Moreover, ELISA is time consuming, requires technical skill and experience to run. Other techniques that detect parasite antigens, such as radioimmunoprecipitation-PEG assays (RIPEGA)

or immunoradiometric assays (IRMA), have the added disadvantage of requiring radioactive reagents. In contrast, the DIA requires only commercially available materials and reagents with long shelf-lives and the test can be assessed visually (13, 14). Our results from earlier studies indicated that detection of parasite antigens in serum by the DIA was as specific as microplate ELISA technique but was simpler to perform under field conditions (14, 15). We used 6 ul of RbBmCAg and 6 ul patients' sera (1 : 10 dilution) which were directly dotted onto the NC per dipstick in comparision to 150 ul to 200 ul per well for microplate ELISA. The actual concentration of antibodies bound per well of the microtiter plate after washing is however unknown and the possibility exists that some may bind weakly or not at all to the well surfaces due to differences in their binding characteristics. Well surfaces have been shown to vary greatly between different brands of microtiter plates. These potential problems can be minimized by applying antibodies on nitrocellulose membrane, which binds antibodies avidly.

The DIA is a rapid test and can be read in approximately 2 hours. As the reaction developed into blue dots, positivity can be deduced visually even by ordinary staff. Positive detection of antigenemia indicate active infection. Additional advantages of parasite antigen detection are that serum antigen levels are believed to be correlated with infection intensity and that antigen levels are constant throughout the day and independent of microfilarial periodicity (9, 10, 11). The significance of parasite antigenemia in asymptomatic, amicrofilaremic individuals is unclear at present.. We have found that most individuals in this category are amicrofilaremic even by membrane filtration (authors' unpublished observations). We believe that a positive antigen test in these amicrofilaremic immigrants indicate the presence of subclinical infection since they were all from countries which are endemic for lymphatic filariasis. An additional advantage of the DIA test for filarial antigenemia which we have developed was that all assay steps were performed at room temperature, enhancing the facility of DIA for field application. Additional studies are needed to explore this possibility.

We would like to emphasize that while parasite antigen detection is sensitive indicator of infection, it is not a substitute for microfilaria detection. Obviously microfilaremia is an important determinant of the infectivity of an individual and the transmission potential of filariasis in populations. However, since antigen testing identifies essentially all microfilarial carriers, one could consider using strategy of screening blood collected during the day by this rapid DIA to determine infection prevalence and intensity. Night blood examinations could then be limited to those with positive antigen tests to determine microfilarial prevalence rates and levels.

Lymphatic filariasis is endemic in Indonesia and Bangladesh. In Malaysia *W. bancrofti*, especially in the cities have been eliminated. However, the vector that transmit urban *W. bancrofti* is still in abundance in the cities of Malaysia. With the influx of immigrants especially those infected with *W. bancrofti* and in relation to their occupational nature, *W. bancrofti* may eventually be introduced into the Malaysian community and may change the whole facet of the disease in Malaysia.

Acknowledgements

This study was supported by a research grant of the Ministry of Science, Technology and Environment, Kuala Lumpur, Malaysia, technical identification IRPA 06-02-02-0004. We also thank Mr. Chin Yew Aik, Manager of Ladang Biji Benih RISDA and the staffs of Bahagian Estate Pekebun Kecil (ESPEK), RISDA, Hulu Trengganu, Trengganu Darul Iman, Malaysia.

Correspondence :

Professor Wan Omar Abdullah,
Department of Clinical Laboratory Sciences,
Faculty of Medicine and Health Sciences,
Universiti Putra Malaysia, 43400 UPM,
Serdang, Selangor, Malaysia

References

1. Ottesen, E.A., Duke, B.O. L, Karam, M. and Behbehani, K. Strategies and tools for the control/ elimination of lymphatic filariasis. *Bull. Wld. Hlth. Org.* 1997; **75**: 491-503.
2. Michael, E., Bundy, D.A. and Grenfell, B.T. Re-assessing the global prevalence and distribution of lymphatic filariasis. *Parasitol*. 1996; **112**: 409-428.
3. Evans, D.B., Gelband, H. and Vlassof, C. Social and economic factors and the control of lymphatic filariasis: a review. *Acta Tropica* 1993; **53**: 1-26.
4. Ottesen, E.A and Ramachandran,C.P. Lymphatic filariasis infection and disease: control strategies. *Parasitology Today* 1995; **11**: 129- 131.

5. Lim, PKC and Sim, BTL.. Laboratory techniques in filariasis. Bull. *Inst. Med. Res.* 1983; **19**: 95-104.

6. Eberhard, M.L and Lammie, P.J. Laboratory diagnosis of filariasis. *Clin. Lab. Med.* 1991; **11**: 977-1010.

7. Weil, G.J, Lamie, P.J, Richard, F.O. and Eberhard, M.L Changes in circulating parasite antigen levels after treatment of bancroftian filariasis witrh diethylcarbamazine and ivermectin. *J. Infect Dis.* 1991; **164**: 814-816.

8. Chanteu S., Glaziou, P., Moulia-Pelat, C., Plichart, C., Luquiaud, P. and Cartel J.L. 1994. Og4C3 circulating antigen: A marker of infection and adult worm burden in Wuchereria bancrofti filariasis. *J. Inf. Dis.* 1994; **170**: 247-250.

9. Wan Omar, A. and Hammerberg, B. 1989. A monoclonal antibody-defined carbohydrate antigens of filariids: detection of antigen in various hosts with diverse clinical manifestations. *Trop. Biomed.* 1989; **6**: 13 – 20.

10. Wan Omar, A., Zahedi, M., Hashim, Y. and Sulaiman, O. Detection of *Brugia malayi* antigens in serum samples of humans living at Perak Tengah, Peninsular Malaysia: Field evaluation. Jpn. *J. Sanitory Zool.* 1994; **45(2**): 147-150.

11. Wan Omar, A., Zahedi, M., Hashim, Y. and Mulkit, S. A monoclonal antibody-based antigen test and correlation of antigenemia with worm burden in canine dirofilariasis. *J. Biosci.* 1994; **3**: 21-25.

12. Weil, G.J, Lammie, P.J. and Weiss N. The ICT filariasis test: A rapid format antigen test for diagnosis of bancroftian filariasis. *Parasitol. Today,* 1997; **13**: 401-404.

13. Ramzy, R.M.R, Hafez O.N. and Gada, M. Efficient assessment of filariasis endemicity by screening for filarial antigenemia in a sentinnel population. Trans. Roy. *Soc.Trop. Med. Hyg.* 1994; **88**: 41-44.

14. Wan Omar, A., Low, C.K., Vivian, J.G., Sulaiman, O. and Yusof, S. A colouremetric dipstick colloidal dye immunoassay for rapid diagnosis of active cases of canine dirofilariasis. *Asian Pacific J. Mol. Biol. Biotech.* 1998; **6**: 41-46.

15. Wan Omar, A., Hashiim, Y., Cheong, L.C. and Vivian, G. Sensitivity and optimization of disperse dye immunoassay (DIA) for the detection of parasite antigenemia in lymphatic filariasis. *Proc. 8th National Biotech. Seminar* 1996: 322-325.

16. Gribnau, T., Van Sommeren, A. and Van Dinther, F. 1983. DIA disperse immunoassay. In: Chaiken,M.Wilchek and Parikh, I., eds. Affinity chromatography and Biological recognition: Orlando: *Academic Press*, 1983: 375- 380.