USM J/P-06

Semua laporan kemajuan dan laporan akhir yang dikemukakan kepada Bahagian Penyelidikan dan Pembangunan perlu terlebih dahulu disampaikan untuk penelitian dan perakuan Jawatankuasa Penyelidikan di pusat pengajian

BAHAGIAN PENYELIDIKAN & PEMBANGUNAN

CANSELORI

UNIVERSITI SAINS MALAYSIA

Laporan Akhir Projek Penyelidikan Jangka Pendek

1) **Nama Penyelidik:** DR. RAHMAH NOORDIN

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2. CIK NOOR A'SHIKIN AZAHRI

JUGA TERLIBAT:
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DARI UNIVERSITI MALAYA:
3. DR. ZURAINEE MOHD. NOOR
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2) **Pusat Pengajian/Pusat/Unit :** PUSAT PENGAJIAN SAINS PERUBATAN
JABATAN MIKROBIOLOGI & PARASITOLOGI

3) **Tajuk Projek:** FIELD EVALUATION OF A POTENTIAL INFECTION MARKER FOR BRUGIAN FILARIASIS

4) (a) **Penemuan Projek/Abstrak**

*(Perlu disediakan makluman di antara 100 - 200 perkataan di dalam **Bahasa Malaysia dan Bahasa Inggeris** Ini kemudiannya akan dimuatkan ke dalam Laporan Tahunan Bahagian Penyelidikan & Pembangunan sebagai satu cara untuk menyampaikan dapatan projek tuan/puan kepada pihak Universiti).*

ANTIGEN *BRUGIA MALAYI* YANG SPESIFIK UNTUK PENGESANAN PENYAKIT FILARIA

Analisis blot Western telah dijalankan terhadap 444 sampel serum: 40 sera daripada individu bermikrofilaria, 10 sera daripada pesakit untut dari kawasan endemik *B. malayi,* 24 pesakit filaria yang telah dirawat, 50 sera daripada penduduk kawasan endemik yang tidak mempunyai antibodi anti-filaria IgG4 (endemik normal), 20 sera daripada individu tak-bermikrofilaria yang mempunyai titer tinggi bagi antibodi anti-filaria IgG4 , 200 sera daripada penduduk bandar dan 100 sera daripada penduduk kawasan tak-endemik yang mempunyai jangkitan cacing bawaan tanah.

Sistem elektroforesis Phast telah digunakan untuk elektroforesis antigen cacing dewasa *B. malayi* di atas gel gradien 10-15% SDS-PAGE dan diikuti dengan perpindahan protin secara elektroforesis ke atas kertas membran PVDF. Jalur-jalur membran kemudiannya dieram secara berturut-turut di dalam larutan penutup ('blocking solution'), sera manusia dan antibodi monoklon anti-manusia IgG4-HRP; dengan langkah basuhan diantaranya setiap langkah pengeraman. Hasil tindakbalas antibodi-antigen tersebut dikesan melalui substrat 'luminol chemiluminescence'.

Band antigenik yang berat molekulnya ~37 kDa telah dikesan secara konsisten dalam semua blot Western yang menggunakan sera individu yang bermikrofilaria, semua individu yang tak-bermikrofilaria tetapi mempunyai titer antibodi anti-filarial IgG4 yang tinggi, sesetengah individu terjangkit yang telah dirawat dan sesetengah pesakit untut. Antigen tersebut tidak dikesan dalam blot Western yang menggunakan sera individu yang dijangkiti cacing bawaan tanah, sera endemik normal dan sera penduduk bandar.

Antigen *B. malayi* yang mempunyai berat molekul ~ 37 kDa mempamerkan tindakbalas spesifik terhadap sera individu yang dijangkiti *B. malayi*; dengan itu antigen ini sangat berfaedah untuk aplikasi ujian diagnostik.

A *BRUGIA MALAYI* ANTIGEN SPECIFICALLY RECOGNIZED BY INFECTED INDIVIDUALS

Western blot analyses were performed on 444 serum specimens : 40 sera from microfilaraemic individuals, 10 sera from elephantiasis patients, 24 treated individuals, 50 sera from residents of endemic areas without anti-filarial IgG4 antibodies (endemic normals), 20 sera from amicrofilaraemic individuals with high anti-filarial IgG4 antibodies, 200 sera from healthy city-dwellers (non-endemic samples) and 100 sera from soil-transmitted helminth infected individuals.

Phast Electrophoresis System was used to electrophorese *Brugia malayi* soluble adult worm antigen on 10-15% SDS-PAGE gradient gels followed by electrophoretic transfer onto PVDF membranes. Membrane strips were then successively incubated with blocking solution, human sera and monoclonal anti-human IgG4 antibody-HRP ; with adequate washings done in between each incubation steps. Luminol chemiluminescence detection was then used to develop the blots.

An antigenic band with the ~ MW of 37 kDa was found to be consistently present in the Western blots of all microfilaraemic sera, all amicrofilaraemic sera with high titres of anti-filarial IgG4 antibodies, some treated patients and some elephantiasis patients. The antigen did not occur in immunoblots of individuals with other helminthic infections, normal endemic individuals and city dwellers.

Therefore the *B. malayi* antigen of ~ MW of 37 kDa demonstrated specific reactions with sera of *B. malayi* infected individuals and thus may be useful for diagnostic application.

(b) **Senaraikan Kata Kunci yang digunakan di dalam abstrak:**

Bahasa Malaysia	**Bahasa Inggeris**
BRUGIA MALAYI	BRUGIA MALAYI
ANTIGEN SPESIFIK	SPECIFIC ANTIGEN
JANGKITAN AKTIF	ACTIVE INFECTION

5) **Output Dan Faedah Projek**

(a) Penerbitan *(termasuk laporan/kertas seminar)*
(Sila nyatakan jenis, tajuk, pengarang, tahun terbitan dan di mana telah diterbit/dibentangkan).

PENERBITAN DALAM JURNAL
"BIOCHEMICAL & BIOPHYSICAL RESEARCH COMMUN."

PERHATIAN: E-MAIL BERKENAAN PENERIMAAN
KERTAS-KERJA INI DILAMPIRKAN

(b) **Faedah-Faedah Lain Seperti Perkembangan Produk, Prospek Komersialisasi Dan Pendaftaran Paten:**
(Jika ada dan jika perlu, sila gunakan kertas berasingan)

Daripada keputusan penyelidikan ini, serum-serum tertentu telah dikenalpasti untuk digunakan dalam penyaringan ' cDNA library' *Brugia malayi* (geran jangka panjang IRPA RM 7). Penyaringan tersebut telah menghasilkan penemuan klon yang spesifik. Seterusnya protein rekombinan yang terhasil telah menunjukkan potensi untuk dijadikan ujian diagnostik yang mungkin boleh dikomersilkan. Jika berjaya, penyelidikan jangka pendek ini membantu dalam menghasilkan produk tersebut.

(c) **Latihan Gunatenaga Manusia**

***Pelajar siswazah*:** Seorang pelajar M.Sc dan seorang pelajar Ph. D. telah melibatkan diri dalam projek ini. Dengan itu matlamat pemindahan teknologi dan ilmu telah tercapai.

Pelajar prasiswazah: Seorang pembantu makmal telah bertugas di bawah projek ini.

***Lain-lain*:** Seorang pegawai sains dan seorang penolong pegawai sains kanan juga telah melibatkan diri dalam projek ini. Dengan itu mereka telah dapat meningkatkan/mengembangkan diri dalam bidang penyelidikan.

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6. **Peralatan Yang Telah Dibeli:**

TIADA

UNTUK KEGUNAAN JAWATANKUASA PENYELIDIKAN UNIVERSITI

T/TANGAN PENGERUSI
J/K PENYELIDIKAN
PUSAT PENGAJIAN

Cover page

A *BRUGIA MALAYI* ANTIGEN SPECIFICALLY RECOGNIZED BY INFECTED INDIVIDUALS

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A *BRUGIA MALAYI* ANTIGEN SPECIFICALLY RECOGNIZED BY INFECTED INDIVIDUALS

SUMMARY Western blot analyses were performed on 444 serum specimens : 40 sera from microfilaraemic individuals, 10 sera from elephantiasis patients, 24 treated individuals, 50 sera from residents of endemic areas without anti-filarial IgG4 antibodies (endemic normals), 20 sera from amicrofilaraemic individuals with high anti-filarial IgG4 antibodies, 200 sera from healthy city-dwellers (non-endemic samples) and 100 sera from soil-transmitted helminth infected individuals. Phast Electrophoresis System was used to electrophorese *Brugia malayi* soluble adult worm antigen on 10-15% SDS-PAGE gradient gels followed by electrophoretic transfer onto PVDF membranes. Membrane strips were then successively incubated with blocking solution, human sera and monoclonal anti-human IgG4 antibody-HRP ; with adequate washings done in between each incubation steps. Luminol chemiluminescence detection was then used to develop the blots. An antigenic band with the ~ MW of 37 kDa was found to be consistently present in the Western blots of all microfilaraemic sera, all amicrofilaraemic sera with high titres of anti-filarial IgG4 antibodies, some treated patients and some elephantiasis patients. The antigen did not occur in immunoblots of individuals with other helminthic infections, normal endemic individuals and city dwellers. Therefore the *B. malayi* antigen of ~ MW of 37 kDa demonstrated specific reactions with sera of *B. malayi* infected individuals and thus may be useful for diagnostic application.

Lymphatic filariasis caused by *Brugia malayi* and *Brugia timori* affects about 12.5 million people in South East Asia (1) and is still endemic in several states in Malaysia. The routine diagnostic test employed to detect brugian filariasis is by night blood examination for microfilaria. This method is specific but insensitive (2) and require night blood sampling which is unpopular with patients, communities and health workers. A more sensitive assay which obviate night blood sampling is required; thus this study was aimed at identifying *B. malayi* antigen that may be specific for serological diagnosis of brugian filariasis.

MATERIALS AND METHOD

Antigen preparation

Gerbils were infected intraperitoneally with the third stage larvae of *B. malayi*. After 3-4 months, the animals were dissected for collection of adult worms. The worms were washed, cut, homogenised, sonicated and freeze-thawed. The preparation was then concentrated, centrifuged and the supernatant kept (-20^{0}C) as soluble *B. malayi* antigen. The protein content of the antigen was determined to be 2000 µg/ml by the Bio-Rad assay.

Study population

Sera from a brugian filariasis endemic area in two states in North Eastern Malaysia was used for this study. Informed consent was obtained from all subjects before the blood sampling. Microfilaria detection was performed using stained thick blood smear and Knott concentration technique. A sandwich ELISA to detect anti-filarial IgG4

antibodies in sera from this area had previously been performed and the cut-off optical density value for detection of active infection was determined to be 0.420 (3). 444 serum samples from the following groups of individuals were employed: 1. Individuals with circulating microfilaria (n = 40) 2. Amicrofilaraemic individuals with elephantiasis (n = 10). 3. Previously microfilaraemic individuals i.e. they have been treated and were amicrofilareamic at time of sampling (n = 24) 4. Endemic normals i.e. endemic area individuals who demonstrated no anti-filarial IgG4 antibodies towards adult worm and microfilaria antigens (previously determined; n = 50). 5. Amicrofilaraemic individuals in endemic areas with high titres of anti-filarial IgG4 antibodies (n = 20). 6. City-dwellers (non-endemic samples; n = 200). 7. Non-endemic area individuals infected with soil-transmitted helminths (STH; n = 100).

Western blotting

Phast Electrophoresis System (Pharmacia, Sweden) was employed for running of the SDS-PAGE gel and for electroblotting. 2 µl antigen per well was electrophoresed on 10-15% gradient gels and the protein bands transferred onto a PVDF membrane. The membrane was then cut into strips, the lane containing the molecular weight marker was stained in amido black and the other strips were washed and incubated in 1% blocking solution (Boehringer Mannheim, Germany) for 30 minutes at room temperature (rt). The strips were then washed in Tris-buffered saline containing 0.05% Tween 20 twice (10 mins/wash) followed by incubation (2 X 10min) in 0.5 % blocking solution. Sera at 1: 200 dilutions (in 0.5% blocking solution) were added to the strips and incubated for 2h, rt. This was followed by a washing step (as above) and the strips were then incubated with monoclonal anti-human IgG4 conjugated to

horseradish peroxidase (CLB, Netherlands) at 1: 1000 for 30 minutes. Another washing step followed, then the blots were developed using luminol chemiluminescence blotting substrate (Boehringer Mannheim). The approximate molecular weights (~ MW) of the antigenic bands were determined by a digital image analyzer (IS-1000 Alpha Innotech Corporation, USA).

RESULTS

There were about 10-12 major antigenic bands in the blots of microfilaraemic individuals, the ~ MWs are 15, 19, 22, 28, 30, 37, 40, 42, 56, 62, 73 and a few bands greater than 96 kDa. A majority of the antigenic bands appeared when sera of both infected and non-infected individuals (especially those with STH infections) were employed. However, an antigenic band with ~ MW of 37 kDa showed a specific appearance in the Western blots of infected individuals used i.e. all microfilaraemic sera (40/40) and all endemic area individuals with high anti-filarial IgG4 antibody titres (20/20). The band was also observed in some chronic elephantisis cases (3/10) and half of the treated patients (12/24).

However, this antigen was absent in the blots probed with the following sera: city-dwellers (200/200), STH patients (100/100), endemic normals with no anti-filarial IgG4 antibodies (50/50), some sera of chronic elephantiasis patients (7/10) and 50% of the treated patients (12/24). Figure 1 shows representative results of this study.

DISCUSSION

Improved diagnosis of lymphatic filariasis is now possible by antigen detection tests for bancroftian filariasis (4, 5) and polymerase chain reactions for both brugian and bancroftian filariasis (6, 7). Diagnosis based on nucleic acid amplification is very specific and sensitive but may not diagnose amicrofilaraemic cases, is more expensive and technically demanding. Therefore a diagnostic assay for brugian filariasis that is cheaper and will diagnose amicrofilaraemic patients is required.

Western blot analysis of native and recombinant *B. malayi* antigens have been reported by several investigators (8, 9, 10 11, 12). However there was no demonstration of an antigen that is very specific for diagnosis of *B. malayi* infection.

The presence of anti-filarial IgG4 antibodies has been strongly correlated to active infection in lymphatic filariasis (3, 13). This study demonstrated that the *B. malayi* adult worm antigen of ~ MW 37 kDa recognised by anti-filarial IgG4 antibodies is potentially useful as a marker of infection. The demonstration that sera of microfilaraemic individuals and amicrofilaraemic individuals with high titres of the anti-filarial IgG4 antibody were reactive to the antigen; and that sera of non-infected individuals (city-dwellers, STH patients, endemic normals) were not reactive, strongly suggest the specificity of the antigen for detection of *B. malayi* infection.

Elephantaisis is a late stage lymphatic filaria infection, thus in most patients the adult worms are dead or dying. Thus patients in this category who demostrated the antigen may harbour dying adult worms which will continue to elicit antibody production, thus

may explain the presence of the antigenic band in 30% (3/10) of the blots of elephantiasis patients. Half (6/12) of the treated individuals demonstrated the antigen; this is expected since they were treated at different times and anti-filarial antibodies will persists for several months to a year (or more) post-treatment. Likewise circulating *Wuchereria bancrofti* antigen test remain positive months after therapy (4). Moreover, the drug causes some adverse reaction such as headache, nausea, vomiting, weakness; thus treatment non-compliance could be a factor; or these individuals could be reinfected.

In conclusion, this study demonstrated that the adult *B. malayi* antigen with ~ MW 37 kDa is a potential candidate for the specific serodiagnosis of brugian filariasis. To enable production of sufficient amounts of this antigen for further studies, a cDNA library of *B. malayi* adult worm is now being screened for expression of this protein.

ACKNOWLEDGEMENTS

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Legends to Figure 1

IgG4 - blots of *Brugia malayi* adult worm antigen using sera from various groups of individuals

1, 2 : blots using sera from two microfilareamic individuals

3 : blot using serum from an amicrofilaraemic individual with high levels of anti-filarial IgG4 antibodies

4,5 : blots using sera from two treated filarial patients, with and without reactivity to the 37 kDa adult *B. malayi* antigen respectively

6, 7 : blots using sera from two elephantiasis patients, with and without reactivity to the 37 kDa adult *B. malayi* antigen respectively

8, 9 : blots using pooled sera (10 from each group) from city dwellers and endemic normals respectively

10, 11, 12 : blots using sera from non-endemic area individuals infected with *Ascaris lumbricoides*, *Trichuris trichiura* and hookworm respectively

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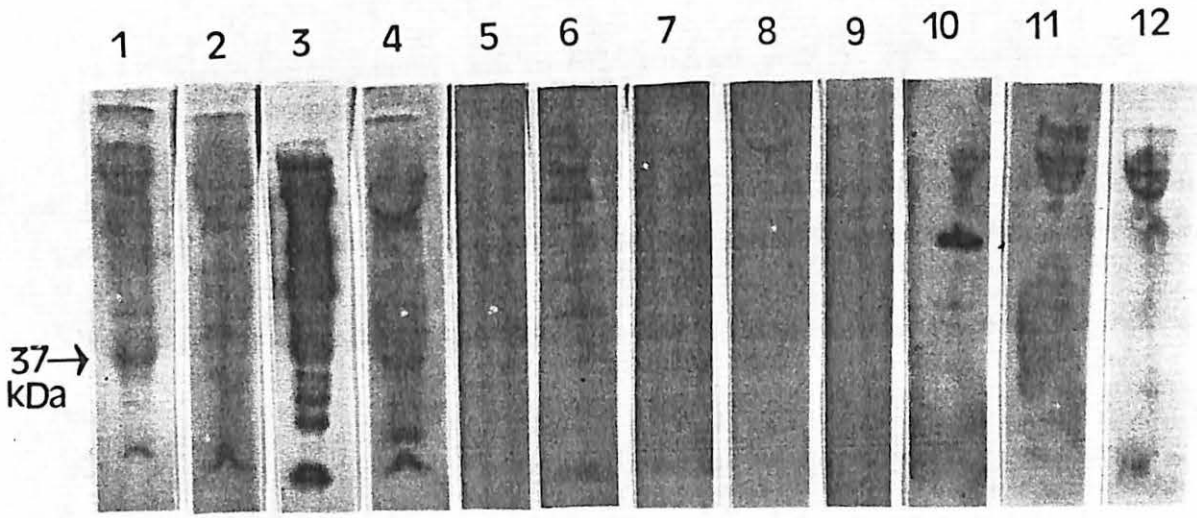
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