Seroreactivity of purified \textit{Brugia malayi} microfilarial soluble and excretory-secretory antigens in different clinical presentations of bancroftian filariasis

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\textit{Brugia malayi} microfilarial excretory-secretory (mf ES) and phosphate buffer saline soluble (mf S) antigens were fractionated by fast protein liquid chromatography (FPLC) on superdex 200 HR 10/30 gel filtration column. The active antigen fractions were identified and explored in comparison with whole mf ES and mf S antigens to detect filarial IgG antibodies in different groups viz microfilaraemics, acute, chronic and occult filarial cases of \textit{Wuchereria bancrofti} infection and endemic and non-endemic normals. One of the fractions of mf ES antigen (ESF-6) and two fractions of mf S antigen (SF-2 & 3) were identified to be useful to detect filarial antibodies. A pooled preparation of these antigen fractions gave a sensitivity of 86.6\% (for microfilaraemic cases) and a specificity of 95\% to detect filarial IgG antibodies by indirect ELISA. The pooled FPLC purified mf antigens also showed 55-88\% of cases of different grades of clinical filariasis and 65\% of tropical pulmonary eosinophilia cases as positive for filarial antibodies. The pooled FPLC purified \textit{B. malayi} mf antigens with higher specificity are preferable to whole mf ES and mf S antigens to detect active filarial infection in microfilaraemia and as well in different clinical entities of bancroftian filariasis.

One of the essential requirements for the effective management of lymphatic filariasis is the availability of a suitable diagnostic test, which could be useful for detection of active infection in individual cases, determining the level of endemicity and evaluation and monitoring of control programmes. The conventional diagnostic approach based on identification of microfilaria in night blood smear is inconvenient and not sensitive enough to detect low microfilaraemics\textsuperscript{1,2}. Moreover microfilariae are not usually seen in the peripheral circulation in acute, chronic and occult filariasis\textsuperscript{3}. Immunodiagnostic assays based on detection of parasite antigens or antibodies against them in circulation have been developed\textsuperscript{4,4}. Filarial antigen assays are suggested to be superior to the assays based on detection of filarial antibodies for indication of current infection\textsuperscript{5}. While filarial antigen assays are quite useful to detect microfilaraemics, they usually gave negative results for amicrofilaraemic subjects with clinical filariasis\textsuperscript{5-7}. Filarial antibodies, on the other hand, have been detected across the clinical spectrum of filariasis\textsuperscript{8-10} and the specific antibody assays were found to be useful to detect and monitor the disease in acute, chronic and occult\textsuperscript{1} filarial cases. \textit{Brugia malayi} microfilarial antigens have been reported to be potentially useful in the detection of \textit{Wuchereria bancrofti} infection\textsuperscript{11,12}. In the present communication, we have fractionated \textit{B. malayi} mf excretory-secretory (ES) and phosphate buffer saline (PBS) soluble antigens, identified active fractions and explored their diagnostic use in different clinical presentations of bancroftian filariasis.

**Materials and Methods**

\textit{Human sera}—Blood samples were collected from 30 microfilaraemic and 65 clinical filarial cases in Sevagram and surrounding villages in Maharashtra (India), which are endemic for nocturnally periodic \textit{W. bancrofti}. The presence of mf was checked in the finger prick blood samples collected at night between 21.00 and 24.00 hours. The chronic filarial cases grouped as described earlier\textsuperscript{13} included 25 of grade I (with acute episodes of filarial lymphangitis, lymphadenitis with or without fever or oedema for 1 to 3 months), 20 of grade II (with oedema or hydroucele for 2 to 5 years, several acute episodes of lymphangitis) and 20 of grade III (with established elephantiasis or hydroucele for 10 to 30 years). The occult filariasis group consisted of 20 cases of tropical pulmonary eosinophilia (TPE). Circulating microfilaria were not found in any of the patients with clinical filariasis. Blood samples were also collected from 30 healthy individuals (endemic
normals) who had lived in endemic regions for over five years and had no history of filariasis and 10 healthy individuals (non-endemic normals) who stay in filarial non-endemic areas (states of Jammu & Kashmir, Haryana, Punjab and Himachal Pradesh in India). Sera were separated and stored at -20°C with sodium azide as preservative.

**B. malayi** microfilarial excretory-secretory (Bm mf ES) antigen—**B. malayi** microfilariae (mf) were obtained from laboratory infected jirds (Marionales unguiculatus) as described by Chenthamarakshan et al. The mf were maintained in RPMI 1640 medium supplemented with organic acids and sugars of Grace's insect culture medium and 0.1 mM phenyl methyl sulphonyl fluoride (PMSF) at 28°C for 48 hr. The supernatant was collected, concentrated by ultramembrane filtration and dialysed against 0.05M sodium phosphate buffer (SPB) pH 7.2. The protein content was measured by Lowry's method and the antigen was stored at -70°C with a cocktail of proteases inhibitors as described by Reddy et al.

**B. malayi** microfilarial soluble (mf S) antigen—B. malayi mf antigen was prepared as described by Kaliraj et al. with few modifications. About 10 lakhs mf were washed twice with 0.05M phosphate buffer saline (PBS) pH 7.2. The mf pellet was suspended in 2ml of 0.05 M PBS containing 0.1mM of ethylene diamine tetra acetie acid (EDTA) and Tosyl L-lysine chloromethyl ketone (TLCK) and homogenised at 4°C for 30 min. The homogenate was further sonicated (using Vibronics sonicator), 10 times each time for 30 sec at 1 min intervals and further extracted overnight by gentle shaking at 4°C. The extract was centrifuged at 13000 g for 30 min at 4°C and the supernatant collected was labelled as Bm mf S antigen. After estimation of proteins the antigen was stored at -70°C until further use.

**Fractionation of B. malayi mf antigens by fast protein liquid chromatography (FPLC)**—Bm mf antigens were fractionated by FPLC using superfine 200 HR 10/30 gel filtration column (Pharmacia Biotech, Sweden) following the manufacturer's instructions. Mf ES antigen (250 µg/0.5ml) and mf S antigen (300 µg/0.5 ml) were applied and eluted using 0.05 M PBS pH 7.2 at a flow rate of 0.5 ml/min and monitored at 280 nm. Fractions of 2 ml volume were collected and the fractions of individual protein peaks were pooled and checked for antigen activity by indirect ELISA using referral pooled filarial positive and negative sera. Thyroglobulin (669 kDa), ferritin (440 kDa) catalase (232 kDa), aldolase (158 kDa), human albumin (66 kDa), lysozyme (14.3 kDa) of Pharmacia Biotech, Sweden were used as molecular weight standards to calibrate the column.

**Indirect enzyme linked immunosorbent assay (ELISA)**—Indirect ELISA was performed as described by Reddy et al. to evaluate the whole and FPLC purified B. malayi mf antigens in detection of filarial IgG antibodies in human sera. The wells in polyvinyl chloride (PVC) microtitre plates (Dynatech, U.S.A.) coated with optimal concentrations of Bm mf S antigen (200 ng/50µl/well), Bm mf ES antigen (25ng/50µl/well) and FPLC fractions (diluted 30-fold), optimally diluted human sera (1:100) and antimouse IgG-peroxidase conjugate were used. The results obtained by using orthophenylene diamine (OPD) as substrate were recorded as optical density (OD) values read at 490nm using Multiscan ELISA Reader (ECIL, India).

**Results**

Fractionation of Bm mf ES and mf S antigens by FPLC using superfine 200 HR 10/30 gel filtration column yielded 6 protein fractions each as shown in Fig. 1A & B. The results of analysis of these fractions for their reactivity with referral pooled filarial and healthy normal sera are shown in Fig. 2 A & B. One of the fractions of mf ES antigen (ESF-6) and three fractions of mf S antigen (SF-2, 3 and 4) were significantly more reactive with referral pooled filarial serum than with referral pooled endemic normal serum.

The antigen fractions ESF-6 and SF-2, 3 and 4 were used to screen individual sera samples (30 from each group) of microfilaraemia, clinical filariasis and endemic normals (Table 1). While the SF-4 antigen showed only 12 (43%) microfilaraemic sera as positive, the other three antigens (ESF-6, SF-2 and 3) showed 20 (66.6%), 23 (76.6%) and 21 (70%) respectively of these samples as positive for filarial antibodies. Of the 30 clinical filarial sera, filarial IgG antibodies were detected in 24 (80%) sera using ESF-6, in 22 (73%) sera using SF-2 antigen and in 20 (66.6%) sera using SF-3 and SF-4 antigens.

A pooled preparation of FPLC purified ESF-6, SF-2 and SF-3 antigens was evaluated in comparison with whole mf ES and mf S antigens for its diagnostic use in bancroftian filariasis based on detection of filarial IgG antibodies (Table 2). None of the 10 nonendemic normal sera samples were positive with
any of the three antigens used. While, mf ES and mf S antigens showed 23% and 30% of 30 endemic normals as positive respectively, pooled specific FPLC fractions (ESF-6, SF-2 and SF-3) showed only 6.6% of them as positive for filarial antibodies. Using mf ES antigen 21 (70%) of 30 microfilaraemic sera were positive while both mf S antigen and pooled FPLC fractions (ESF-6, SF-2 and SF-3) showed 26 (86.6%) sera as positive. The positivity in the clinical and occult filarial sera varied broadly (55-88%) depending on the stage of infection and type of antigen used.

Discussion

The limitations associated with parasitological tests have necessitated the development of suitable immunodiagnostic tests for filariasis. A number of filarial antigen assays have been reported and shown to be quite useful to detect actively infected cases particularly microfilaraemic. It has also been noted that amicrofilaraemics with clinical manifestations are usually negative for filarial antigen using most of these assays. Demonstration of antibodies against specific filarial antigens is useful in this context to detect active infection in such cases and for their better management. In the present study, we have evaluated the use of a cocktail preparation of specific FPLC purified B. malayi mf antigens (ESF-6, SF-2 and SF-3) to detect filarial IgG antibodies across the clinical spectrum of filariasis.

By FPLC fractionation of B. malayi mf ES and mf S antigens, it was possible to identify four active antigen fractions (ESF-6, SF-2, 3 and 4) having higher reactivity with pooled filarial serum than with pooled endemic normal serum (Fig. 2). Of these four antigen fractions, when further tested for screening individual sera, SF-4 antigen showed only 43%
microfilaraemic sera as positive, while the other three antigens (ESF-6, SF-2 and SF-3) gave 66.6-80% positivity for microfilaraemic and clinical filarial sera (Table I).

Hence a pooled preparation of ESF-6, SF-2 and SF-3 antigens was evaluated for its diagnostic use in detection of active filarial infection. The results are compared with those of whole Bm mf ES and mf S antigens (Table 2). Pooled specific FPLC purified antigens gave a sensitivity of 86.6% (in detecting filarial IgG antibodies in mf positive cases), which is similar to that of whole mf S antigen and higher than that of whole mf ES antigen (70%). Pooled purified antigen fractions gave higher specificity (95%) compared to the specificity obtained by using whole mf ES antigen (82.5%) or whole mf S antigen (77.5%). Further, using pooled specific FPLC purified antigens, filarial IgG antibodies were detected in 55-88% of different grades of clinical filariasis and 65% of occult (TPE) cases (Table 2) and these results were better than using either mf ES or mf S antigen alone. ELISA tests using a combination of antigens has been shown to achieve higher sensitivity and specificity in diagnosis of tuberculosis.\(^\text{20}\)

\(W.\) \textit{bancrofti} mf soluble antigen has been used by Kaliraj \textit{et al}.\(^\text{21}\) to detect filarial antibodies by indirect haemagglutination test (IHAT), indirect fluorescent antibody test (IFAT) and ELISA. As many as 45-65% endemic normals were reported to be positive for filarial antibodies in these tests. The saline extracts of \(B.\) \textit{malayi} mf, adult and infective larvae were used in skin test to detect \(W.\) \textit{bancrofti} infected cases.\(^\text{22}\) \(W.\) \textit{bancrofti} mf ES antigens have been earlier used by

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**Table 1** — Detection of filarial IgG antibodies in different groups of sera using FPLC fractions of \(B.\) \textit{malayi} microfilarial antigens in indirect ELISA

<table>
<thead>
<tr>
<th>Sera</th>
<th>No. Exam.</th>
<th>No. Positive* (% positivity) using FPLC purified filarial antigen</th>
</tr>
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<tbody>
<tr>
<td>a) Endemic normals</td>
<td>30</td>
<td>ESF-6: 5 (16.6), SF-2: 3 (10), SF-3: 4 (13.3), SF-4: 5 (16.6)</td>
</tr>
<tr>
<td>b) Microfilaraemia</td>
<td>30</td>
<td>ESF-6: 20 (66.6), SF-2: 23 (76.6), SF-3: 21 (70), SF-4: 12 (43)</td>
</tr>
<tr>
<td>c) Clinical filariasis</td>
<td>30</td>
<td>ESF-6: 24 (80), SF-2: 22 (73.3), SF-3: 20 (66.6), SF-4: 20 (66.6)</td>
</tr>
</tbody>
</table>

* Sera showing O.D. of \(\geq\) mean +2 SD of O.D. of endemic normal sera

**Table 2** — Detection of filarial IgG antibodies in different groups of sera using pooled FPLC fractions of \(B.\) \textit{malayi} mf antigens

<table>
<thead>
<tr>
<th>Group</th>
<th>No. Exam.</th>
<th>ES antigen</th>
<th>PBS (S) antigen</th>
<th>Pooled FPLC fractions**</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Microfilaraemia</td>
<td>30</td>
<td>21 (70)</td>
<td>26 (86.6)</td>
<td>26 (86.6)</td>
</tr>
<tr>
<td>2. Endemic normal (EN)</td>
<td>30</td>
<td>7 (23)</td>
<td>9 (30)</td>
<td>2 (6.6)</td>
</tr>
<tr>
<td>3. Non-endemic normal (NEN)</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4. Grade I Chronic</td>
<td>25</td>
<td>20 (80)</td>
<td>18 (72)</td>
<td>22 (88)</td>
</tr>
<tr>
<td>5. Grade II Chronic</td>
<td>20</td>
<td>13 (65)</td>
<td>13 (65)</td>
<td>16 (80)</td>
</tr>
<tr>
<td>6. Grade III Chronic</td>
<td>20</td>
<td>11 (55)</td>
<td>10 (50)</td>
<td>11 (55)</td>
</tr>
<tr>
<td>7. Occult (TPE)</td>
<td>20</td>
<td>12 (60)</td>
<td>10 (50)</td>
<td>13 (65)</td>
</tr>
</tbody>
</table>

**Sensitivity**

(For mf-ve Sera) 70% 86.6% 86.6%

**Specificity**

(with EN & NEN sera) 82.5% 77.5% 95%

* Sera showing O.D. of \(\geq\) mean +2SD of O.D. of endemic normal sera

** Pooled preparation of FPLC fractions ESF-6, SF-2 and SF-3

TPE=Tropical pulmonary eosinophilia
Harinath et al. detected filarial IgG antibodies with a sensitivity and specificity of 80-90%. B. malayi mf ES antigens have been reported to be useful to detect filarial infection in microfilaraemia, clinical and occult cases and in their long term follow up to determine the optimal period of treatment with diethyl carbamazine citrate (DEC). Chenthamarakshan et al. used a partially purified B. malayi mf ES antigen and reported 70%, 85% and 28% of chronic filarial, microfilaraemic and endemic normals as positive for filarial antibodies. Separation of occult cases and in their long term follow up to establish filarial aetiology in occult cases which turn helps in their better management.

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References


