Kinetics of microfilaraemia & antigenaemia status by Og$_4$C$_3$ ELISA in bancroftian filariasis

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Received June 15, 2005

**Background & objectives:** Bancroftian filariasis caused by *Wuchereria bancrofti* is endemic in many parts of India. In recent years diagnosis of *W. bancrofti* infection has been revolutionized with the availability of filarial antigen tests, which is important in monitoring success of chemotherapy. We carried out this study to measure microfilariaemia and antigenemia levels in bancroftian microfilariae (mf) carriers at 1 yr follow up after chemotherapy, in lymphoedema patients and in endemic controls from a filariasis endemic area in Tamil Nadu State using Og$_4$C$_3$ ELISA to identify the best marker to assess success of chemotherapy.

**Methods:** Serum samples were collected from 30 bancroftian microfilaremic (Mf) carriers pre-treatment and at sequential intervals (7,30,60,90,180 and 365 days) following treatment with diethylcarbamazine (DEC:6mg/kg body weight, single dose), 30 lymphoedema patients (without treatment) at periodic intervals, and 68 control subjects (24 endemic normal subjects in filariasis endemic area in Tamil Nadu State, 24 non-endemic normal subjects residing in Chandigarh, India; 5 brugian filariasis, 5 endemic control subject in brugian filariasis endemic area and 10 other disease controls). The circulating antigen of *W. bancrofti* was measured quantitatively using Og$_4$C$_3$ ELISA kit.

**Results:** In Mf carriers, there was no significant difference in microfilariae count in pre- and post-treatment (PT) samples till day 30 while significant differences were observed in pre- and sequentially collected post-treatment (PT) samples day 60 to 180 ($P<0.001$), day 365 ($P<0.005$). However, there was no significant difference in antigenaemia levels between pre-treatment (day 0) and PT samples collected on day 7 onwards till day 365. Though of the 19 patients who could be followed up till 365 days PT, 4 (21%) were amicrofilaraemic, none became antigen negative. No significant difference was found in antigenaemia levels in sequentially collected samples from lymphoedema patients. Significant differences were observed in antigenaemia levels in samples collected at the start of study in mf carriers as compared to lymphoedema patients and endemic normal subjects ($P<0.001$). Subjects (non-endemic control) residing in filariasis free area (24), brugian endemic area (5), *B.malayi* infected patients (5) and patients with other parasitic diseases (10) were found antigen negative.

**Interpretation & conclusions:** Annual single dose of DEC therapy alone may not result in complete clearance of infection and detection of antigenaemia rather than microfilaraemia may be taken into consideration as an indicator of successful chemotherapy. The study supports the earlier view that filarial antigenaemia is relatively common in amicrofilaraemic and asymptomatic subjects in endemic areas and further studies are needed to determine the clinical significance, prognosis and effective management of such infections in endemic areas.

**Key words** Antigen - bancroftian filariasis - microfilaraemia - Og$_4$C$_3$ ELISA - treatment - *Wuchereria bancrofti*
Human lymphatic filariasis caused by *Wuchereria bancrofti*, *Brugia malayi* and *B. timori* affects approximately 120 million people, with one billion considered to be at risk of becoming infected in 76 countries through regions of South and Central America, West and East Africa, Eastern Mediterranean, Southeast Asia and Western Pacific. *W. bancrofti* accounts for approximately 90 per cent of all filariasis cases in the world. In India, there are approximately 21 million people with symptomatic filariasis and 27 million microfilariae (mf) carriers. Traditionally, it has been accepted that three groups of people are found in a filarial-endemic area: (i) those who are exposed, but with no evidence of disease - so called 'endemic normals', (ii) those with 'asymptomatic microfilariaemia', and (iii) those with chronic lymphoedema, hydrocele and elephantiasis. Recent studies with ultrasonography have shown that 'asymptomatic microfilariaemia' is not a benign phase and that considerable occult lymphatic tissue and organ damage may be occurring. Freedman proposed a new classification: asymptomatic infected individuals who are antigen negative, individuals who have overt filariasis and active infection (antigen positive) and individuals who have overt filariasis without active infection (antigen negative).

In recent years, the diagnosis of *W. bancrofti* has been revolutionized by the introduction of filarial antigen tests. These tests are not dependent upon the presence of microfilariae, blood can be taken at any time and amicrofilaraemic cases could be thus detected. The commercial ELISA, Trop Bio Og4 C3 Antigen Test is based on the assay developed by More and Copeman and is regarded as a good marker of active filarial infection with adult worms. Diethylcarbamazine (DEC) has been used antifilarial drug since 1947 and till date is the most widely chemotherapeutic response.

In the present study we evaluated the microfilariaemia and antigenaemia levels in bancroftian microfilariaemic carriers at sequential intervals till 365 days following DEC treatment, in lymphoedema patients (without treatment) at periodic intervals and in endemic normal subjects from another endemic area of bancroftian filariasis in Tamil Nadu State in India with highly sensitive Og4 C3 ELISA test, to identify the best possible marker for assessing chemotherapeutic response.

**Material & Methods**

**Study area**: The study area included village Alagramam, primary health centre Muppuli, District Villupuram in Tamil Nadu State, India. The population was 3279 with mf rate 17.2 per cent and disease rate 14.2 per cent. The main occupation of the subjects is mainly agriculture labour/weaver.

**Subjects and samples**: Blood samples were collected during 2000-2001 at Vector Control Research Centre (VCRC), Puducherry, from 85 subjects (microfilariae carriers, lymphoedema patients and endemic normal subjects) residing in the above mentioned study area and selected at random at the start of the study (day 0). Examination of blood for mf was done as described earlier. In mf carriers, 60 µl of blood from each subject was examined by thick smear technique. In endemic normal subjects, thick smear examination followed by membrane filtration concentration technique was carried to confirm the mf negative report. Details of age, sex, microfilariae count were recorded at the VCRC, Puducherry. All the serum samples were coded and transported to Postgraduate Institute of Medical Education & Research (PGIMER) Chandigarh, under refrigerated conditions.

**Control samples**: Samples collected at Chandigarh, north India, from 24 normal subjects who were mf negative and did not give any history of travel to any filariasis endemic area during the past five years, were included as non-endemic controls. Serum samples from 5 brugian filariasis patients and 5 endemic normals in brugian filariasis endemic area collected at T.D. Medical College, Alleppey Centre (as part of the ICMR Task Force Project on Filariasis during 2000-2001) and transported to the Department of Parasitology, PGIMER, Chandigarh, and 10 samples from patients with other parasitic diseases (cysticercosis - 2, hydatidosis - 2, malaria - 2, toxoplasmosis - 2, amoebiasis - 2) were included as controls.
Follow up samples: The microfilariae carriers and lymphoedema patients were followed up at VCRC, Pondicherry, for one year. The samples were collected from mf carriers at sequential intervals on 7, 15, 30, 60, 90, 180 and 365 days following DEC treatment (DEC-6mg/kg body weight single dose) and from lymphoedema patients (without treatment) on 90, 180 and 365 days. No follow up samples were collected from endemic normal subjects and non-endemic normal subjects.

Assay for circulating antigen: The circulating antigen of *W. bancrofti* was quantified in all the serum samples (50 µl) using Og 4 C 3 ELISA kit according to the instructions of the manufacturer (JCU Tropical Biotechnology Pvt. Ltd. Queensland, Australia). After boiling pre-treatment, each serum sample was checked in duplicate and the mean optical density (OD) of each sample tested in duplicate was taken as OD reading for that sample. The antigen concentration in units from the standard curve (relating optical density and antigen content of the seven standards enclosed in the kit) was determined as per manufacturer’s instructions.

The study was blind to the extent that the clinical profile, microfilariae status, age and sex details were not known to the staff carrying assay for circulating antigen. After completion of the work, the samples were decoded at the Indian Council of Medical Research (ICMR) headquarters, New Delhi, India.

Statistical analysis: The data analysis was done with the use of SPSS10 software and the following statistical methods were applied to judge the significance of observations: (i) Chi-square and One way ANOVA test was used for age group and gender analysis in different groups, (ii) ANOVA followed by Dunnett procedure for comparison with control (day 0), and Students-Newmann-Kent procedure for pair-wise comparison of microfilariaemia and antigenaemia – Pre- and Post-treatment analysis in mf carriers and at sequential intervals in lymphoedema patients, and (iii) Repeated measure ANOVA for antigenaemia analysis in mf carriers vs lymphoedema patients at different time intervals.

Results

On retrospective analysis, following decoding of the data of the samples received from VCRC, Pondicherry, it was found that 85 subjects from filariasis endemic area comprised of 30 mf carriers and were labeled as Group I, 30 lymphoedema patients as Group II, and 24 endemic normal subjects as Group III. In addition, one subject was found amicrofilaraemic but antigen positive at the start of the study. However, as this subject could not be followed up, so was excluded for statistical analysis. One of the 30 lymphoedema patients was not included at the start of study (day 0) but was subsequently included at day 90 (only one sample at day 90 was collected from this patient).

The samples were collected from 19, 4, 22, 23, 21, 18 and 19 mf carriers on day 7, 15, 30, 60, 90, 180 and 365 days post-treatment (PT) and 21, 17 and 19 lymphoedema patients (without treatment) on 90, 180 and 365 days, respectively.

The subjects in endemic area comprised 48 males and 35 females with mean age of microfilariae carriers, lymphoedema patients and endemic normal subjects as 28.3, 46.9 and 25.5 yr respectively. The maximum number of mf carriers (20 of 30) were in the age group <30 yr, lymphoedema patients (26 of 29) were >30 yr old and endemic normal subjects (17/24) were mainly in the lower age group <30 yr (Table I). Age group and gender analysis indicated significant differences in the

<table>
<thead>
<tr>
<th>Group</th>
<th>Total</th>
<th>M/F</th>
<th>Age groups (yr)</th>
<th>Mean ± SD (Range)</th>
<th>Median age</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>&lt;30  &gt;30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I. Mf carriers</td>
<td>30</td>
<td>23*7</td>
<td>20*10</td>
<td>28.3 ± 12.45</td>
<td>26.5</td>
</tr>
<tr>
<td>II. Lymphoedema</td>
<td>30*</td>
<td>9/20*</td>
<td>326*</td>
<td>46.9±11.27</td>
<td>50.0</td>
</tr>
<tr>
<td>III. Endemic normals</td>
<td>24</td>
<td>16*8</td>
<td>177</td>
<td>25.5 ± 10.28</td>
<td>20.0</td>
</tr>
<tr>
<td>Total</td>
<td>84</td>
<td>48/35</td>
<td>4043</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* In one subject, age and gender information not available
* Significantly more individuals than the other age class/gender within each group (Chi square test)
** Mean age for Group II significantly differed (P<0.001) from Groups I & III whereas there was no difference between Groups I & III (One way ANOVA followed by SNK procedures)
mean age of lymphoedema patients when compared with mf carriers and endemic normals (P<0.001).

Microfilariaemia: Except mf carriers, all other subjects (lymphoedema patients and endemic normals) in endemic area were found to be negative for microfilariae.

Pre- and post-treatment (PT) mf count: In mf carriers, mf count ranged from 1 - 135/60 µl before start of chemotherapy (day 0). Post-treatment samples (PT) could not be collected in 4 of 30 mf carriers. Following DEC treatment 1 of 19 (5.3%) subject on day 7, all 4 (100%) on day 15, 4 of 22 (18.2%) on day 30, 3 of 23 (13%) on day 60, 1 of 21 (4.8%) on day 90, 3 of 18 (16.7%) on day 180 and 4 of 19 (21%) were amicrofilariaemic (Fig. 1). The geometric mean titres were 13.80, 8.42, 6.31, 3.01, 3.05, 3.36 and 3.81 on day 0,7,30,60,90,180 and 365, respectively. No significant differences were observed in counts between pre- (day 0) and post-treatment samples collected at day 7, 15 and 30, while significant differences were observed when compared to day 60,90,180 (P<0.001) and 365 (P<0.005) (Table-II). Out of 19 patients who could be followed up till day 365 post treatment, 4 (21%) became amicrofilaraemic and all the 4 had pre-treatment microfilaraemia of low intensity (3-30/60µl).

Analysis of pre- and post-treatment microfilaraemia between two different age groups (<30 and > 30 yr) indicated no significant differences between pretreatment (day 0) samples compared to PT samples. Similarly, no significant differences were observed in pre- and post-treatment mf counts between males and females (Data not shown).

Antigenaemia:

Microfilariae carriers - The pre-treatment mean antigenaemia levels were 13350.40 units. The geometric mean titres were 3380.26, 4211.65, 726.07, 2620.84, 2592.55, 1208.30, 3495.30 and 1645.80 on

<table>
<thead>
<tr>
<th>Days</th>
<th>Number studied / Mean mf count (SD)</th>
<th>Median</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>30 / 30 (100) 21.40 (26.39)</td>
<td>13</td>
</tr>
<tr>
<td>7</td>
<td>19 / 18 (94.7) 13.47 (15.66)</td>
<td>5</td>
</tr>
<tr>
<td>15</td>
<td>4 / 0 00.00 (00.00)</td>
<td>-</td>
</tr>
<tr>
<td>30</td>
<td>22 / 18 (81.8) 11.14 (14.92)</td>
<td>5</td>
</tr>
<tr>
<td>60</td>
<td>23 / 20 (87) 2.96 (3.35)</td>
<td>2</td>
</tr>
<tr>
<td>90</td>
<td>21 / 20 (95.2) 3.00 (4.40)</td>
<td>2</td>
</tr>
<tr>
<td>180</td>
<td>18 / 15 (83.3) 3.44 (3.91)</td>
<td>2</td>
</tr>
<tr>
<td>365</td>
<td>19 / 15 (78.9) 5.79 (8.71)</td>
<td>2</td>
</tr>
</tbody>
</table>

ANOVA followed by Dunnett procedure for multiple comparisons from day 0 as control

P>0.05 – Day 0 vs 7,30
P<0.001 – Day 0 vs 60,90,180
P=0.005 – Day 0 vs 365

Student – Newman-Kents analysis indicated no significant difference between day 0 and day 7 samples while significant difference was observed between day 0 and day 30 to day 365 collected samples

Fig. 1. Number of microfilamaeric carriers with pre- and post-treatment mf count/60µl.
day 0, 7, 15, 30, 60, 90, 180 and 365 respectively. Although, geometric mean titre decreased from 3380.26 (pre-treatment) to 1645.80 (365 day PT) yet, no significant differences were observed between pre-treatment samples (day 0) when compared to day 7, 15, 30, 60, 90, 180 and 365 PT (Table III). Only 19 mf carriers could be followed up till day 365 PT, none was found antigen negative and levels ranged from 128-32000 units (mean 5559.58) at day 365 PT (Fig. 2). Of the 19 mf carriers who were followed up till day 365 PT, 5 (26.3%) had 4 fold increase in antigenaemia, 9 (47.4%) had decrease while in 5 (26.3%) no significant difference in pre-treatment and day 365 PT samples was observed (individual data not shown).

**Lymphoedema patients** - Mean antigenaemia levels were 38.62, 18.91, 40.47 and 44.63 units and geometric mean titres were 14.25, 11.46, 13.30 and 19.24 on day 0, 90, 180 and 365 respectively. At the start of the study, 24 (82.8%) patients had antigenaemia levels <10 units and 5 (17.2%) had 32-512 units. Of the 19 patients who were followed up till day 365, antigenaemia increased significantly in 8 (42.1%), decreased in 2 (10.5%) and persisted in 9 (47.4%). No significant difference was observed in sequentially collected samples on day 0 vs 90, 180 and 365 and between day 90 vs 180, 365; day 180 vs 365 (Table IV).

**Control subjects** - In 24 endemic normal subjects (asymptomatic and amicrofilaraemic), the mean
antigenaemia levels were 3681 units (geometric mean titre 127.67). Eleven (45.83%) subjects had <10 units and 13 (54.2%) had >32 units. Of the 13 subjects with >32 units, 6 (25%), 5 (20.83%) and 2 (8.33%) had antigenaemia 32-512, >512-8192 and >8192 to 32,000 units respectively. Samples were collected at one time only from each subject.

Non-endemic control subjects, B. malayi infected patients and patients with other parasitic infections were antigen negative.

Comparative analysis of antigen responses in samples collected at the start of study (day 0) in mf carriers, lymphoedema patients and endemic normal subjects indicated highly significant difference ($P<0.001$) between groups (Table V, Fig. 3), and significant differences ($P<0.05$) were observed in antigenaemia levels between mf carriers and lymphoedema patients in follow up samples collected on different days (Table VI).

**Discussion**

Circulating filarial antigen detected by Og$_5$C$_3$ Trop Bio kit is shown to be a sensitive parameter for detecting microfilariae positive subjects. The species specificity of this assay and the lack of fluctuation in antigenaemia compared with microfilaraemia suggest that this assay is suitable for daytime screening$^{13}$. We reported here the pre- and post-treatment (PT) microfilaraemia status in mf carriers and circulating filarial antigen levels in

**Table VI.** Comparative evaluation of antigenaemia (units) between mf carriers (Group I) and lymphoedema patients (Gr II) on different days

<table>
<thead>
<tr>
<th>Time period</th>
<th>Group*</th>
<th>Number</th>
<th>Mean (SD)</th>
<th>Log values mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>I</td>
<td>13</td>
<td>13 774.77 (15244.86)</td>
<td>3.54 (0.932)</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>15</td>
<td>51.20 (131.20)</td>
<td>1.318 (0.530)</td>
</tr>
<tr>
<td>90</td>
<td>I</td>
<td>13</td>
<td>8864.00 (13497.36)</td>
<td>3.126 (1.019)</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>15</td>
<td>16.00 (30.98)</td>
<td>1.031 (0.299)</td>
</tr>
<tr>
<td>180</td>
<td>I</td>
<td>13</td>
<td>7483.08 (11311.01)</td>
<td>3.403 (0.685)</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>15</td>
<td>44.80 (129.52)</td>
<td>1.146 (0.476)</td>
</tr>
<tr>
<td>365</td>
<td>I</td>
<td>13</td>
<td>2217.85 (2773.54)</td>
<td>2.989 (0.675)</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>15</td>
<td>51.20 (128.03)</td>
<td>1.297 (0.480)</td>
</tr>
</tbody>
</table>

$^a$ One way ANOVA and Kruskal-Wallis ANOVA suggested that the groups differed ($P<0.001$)  
$^b$ Students-Newman-Kant procedure- $P<0.05$ compared to Groups II, III  
$^c$ All the three groups differ using MWU test ($P<0.05$)  
$^d$ $P<0.05$ (Repeated measure ANOVA) within groups  
$^e$ $P<0.01$ between groups

![Fig. 3](image-url)
**W. bancrofti** microfilaraemic carriers (before and following DEC treatment), filarial lymphoedema patients (without treatment) and endemic normal subjects (one time sample) in bancroftian filariasis endemic area in India.

Our results on mf counts were consistent with the earlier report from Egypt\(^4\) where though mf counts and antigen levels significantly decreased after treatment, majority of treated subjects were microfilaraemic one year after therapy. Our results were also consistent with the study conducted in Sri Lanka where it was reported that although antigen concentrations were markedly reduced following treatment with a single combined dose of DEC and albendazol (ALB), yet none of the patients became antigen negative during follow up\(^16\).

In the present study, of the 19 mf carriers who were followed up till 365 days PT, 26.3 per cent had significant increase in antigenaemia, 47.4 per cent had significant decrease while in 26.3 per cent no significant difference in pre-treatment and day 365 PT samples was observed. These results were similar to the study from Orissa, India\(^9\) which reported that although all the individuals monitored post DEC- therapy became free from mf, yet 43 per cent had reacquired microfilaraemia and 81 per cent had positive antigenaemia even 10 yr after treatment. The study indicated the presence of adult worms in the majority of treated cases as proportion of mf-negative DEC-treated individuals were Og\(_4\) C\(_3\) antigen positive. However, the possibility of reinfection also exists in the endemic regions\(^9\). The important observation of the present study was that when microfilarial count and antigen levels were compared in PT samples, significant differences were observed in mf count on day 0 as compared to day 60,90,180 and 365 PT while in antigenaemia levels no significant difference was observed in day 0 samples as compared to PT samples. Although, 4(21%) mf carriers (pre-treatment) were amicrofilaraemic at day 365 PT, they were antigen positive, suggesting that antigenaemia detection rather than microfilaraemia should be taken into consideration as an indicator of chemotherapy, for the ultimate control of this infection. These results were consistent with the report from East Africa, where treatment resulted in progressive decrease in microfilaraemia and circulating antigenaemia with relative reductions being considerably higher for mf than for antigenaemia\(^6\).

We found positive antigen response (>32 units) in 17.2 per cent lymphoedema patients at the start of the study (day zero). Earlier studies have reported variable prevalence of antigenaemia in lymphoedema patients\(^5,13\). However, the important observation of the present study was that during the 1 yr follow up period, antigenaemia persisted in 47.4 per cent lymphoedema patients.

Earlier studies in Egypt and India have shown that a significant proportion of asymptomatic and microfilaraemic persons residing in endemic areas have positive filarial antigenaemia levels. The present study conducted in Tamil Nadu, India, an endemic area of bancroftian filariasis, also indicated similar findings. The geometric mean titres at the start of the study were 3380.26, 14.25 and 127.67 respectively for mf carriers, lymphoedema patients and endemic normal subjects. These results were similar to an earlier report\(^13\) where antigen titres in microfilaraemic carriers were found to be significantly higher as compared to antigen positive endemic normals and lymphoedema patients. Further, in the present study, significant differences were observed in antigenaemia levels between mf carriers and lymphoedema patients in sequentially collected samples. None of the non-endemic normal subjects, patients with *B. malayi* and other parasitic infections indicated positive antigen response in the present study, thereby indicating 100 per cent specificity of the Og\(_4\) C\(_3\) ELISA kit.

In conclusion, the findings of our study supported the earlier finding that annual single dose DEC therapy alone may not result in complete clearance of infection in mf carriers\(^19,20\) and detection of antigenaemia rather than mf count should be taken into consideration as an indicator of chemotherapy. Filarial antigenaemia was significantly higher (>32 units) in 54.2 per cent endemic normal subjects and these may be at increased risk for development of clinical disease compared to antigen negative endemic normals (< 10 units in 45.8% subjects). Our study also indicates at the need to evaluate newer drugs with microfilaricidal activity for the ultimate control of this disease.

**Acknowledgment**

Authors acknowledge the Indian Council of Medical Research, New Delhi, for financial support and thank Shrimati Gurjeet Bhatti, Department for Parasitology, for technical assistance and Shri R.C. Goyal, Statistician, Medical Education and Research Cell, PGIMER, Chandigarh, for statistical analysis of data.

**References**


