THE IMPACT OF SINGLE-DOSE DIETHYLCARBAMAZINE TREATMENT OF BANCROFTIAN FILARIASIS IN A LOW-ENDEMICITY SETTING IN EGYPT

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Abstract. This study was designed to evaluate the effect of a single dose of diethylcarbamazine (DEC, 6 mg/kg) on Wuchereria bancrofti infections in a low-endemicity setting in Egypt (microfilariaemia, or MF, 3.7%, median MF 34/mL). Subjects with MF or filarial antigenemia were treated and restudied 1 year later. Treatment with DEC dramatically reduced blood MF counts, with clearance in 69% of subjects. Treatment also reduced filarial antigen levels, but low clearance rates suggest that some adult worms survived treatment in most patients. Mass treatment was administered in one village; 27 months later, MF prevalence had decreased 84% (from 4.9% to 0.8%). These results show that single-dose DEC treatment can have a major effect on MF prevalence rates and levels in low-endemicity settings. Although the World Health Organization advocates repeated multidrug regimens for filariasis elimination, mass treatment with DEC alone may be sufficient to interrupt transmission in areas with low infection intensities and prevalence rates.

INTRODUCTION

Bancroftian filariasis is a deforming and disabling parasitic disease caused by the filarial nematode Wuchereria bancrofti. Reports indicate that bancroftian filariasis is endemic in ~80 countries, with an estimated 100 million people infected. The World Health Organization has initiated the Global Programme for Elimination of Lymphatic Filariasis, which is based on repeated annual cycles of mass treatment of endemic populations with single-dose combinations of albendazole with either diethylcarbamazine (DEC) or ivermectin. This strategy takes advantage of the relative inefficiency of mosquito transmission of filariasis; many infective bites are required to establish an infection. The Global Programme for Elimination of Lymphatic Filariasis aims to interrupt transmission of filariasis by attacking the reservoir of microfilariae, thereby reducing the uptake of parasites by mosquitoes. The use of combination regimens is based on the premise that they are more effective and long-lasting than monotherapy with DEC. However, relatively few studies have been conducted to directly compare single-drug and multidrug regimens for suppression of microfilaraemia (MF). It is also likely that different regimens and schedules of mass treatment could be used for filariasis elimination in areas with different prevalence rates, infection intensities, vector species, environmental conditions, and other factors that affect transmission dynamics.

Mass treatment with repeated, single annual doses of DEC has been utilized for filariasis control in many areas, and this treatment is currently being employed on a large scale in India. Several studies have documented the effects of community-based single-dose DEC on microfilaria prevalence rates and levels. These studies were performed in high-endemicity areas, and they did not employ filarial antigen testing (a measure of adult worm infection intensity) to monitor effects of treatment. The present study was designed to examine the effects of single-dose DEC on MF and parasite antigenemia in an area with low filariasis infection prevalence rates and intensities. The study was conducted in Egypt.

Lymphatic filariasis is focally endemic in Egypt, with an estimated 137,000 people infected and 2 million people at risk for infection, mainly in the Nile Delta region (and Egyptian Ministry of Health and Population [MOHP], unpublished data). The principal mosquito vector responsible for filariasis transmission in Egypt is Culex pipiens pipiens. Although high infection prevalence rates were documented in some areas as recently as 10 years ago, recent surveys show that most endemic areas in Egypt now have low prevalence rates and infection intensities. This may be due to control efforts conducted by the MOHP (mass screening with night blood smears and selective treatment of infected individuals with DEC) and marginal conditions for transmission in some areas. The MOHP initiated a National Filariasis Elimination Program in late 2000 that is based on repeated annual mass administration of single-dose DEC with albendazole in all known endemic localities, with a target population of over 2 million people.

The present study was conducted in 1998–2000, before initiation of the National Filariasis Elimination Program in Egypt. Our results show that single-dose DEC can have dramatic effects on filariasis infection rates and intensities in low-endemicity settings.

PATIENTS AND METHODS

Study population. The study was conducted in 1998–2000 in an area previously studied by our group and known to have low-level filariasis endemicity. The study villages are located ~35 km northeast of Cairo, Egypt, in Shebin El Kanatar District, Qalubia Governorate. Some of the people in these agricultural villages have been screened for filariasis in the past, and many residents have been previously treated for MF with DEC. Recent studies in these villages have shown microfilaria incidence rates of under 1% (and authors’ unpublished observations).

Our 1998 baseline sample comprised 10–20% of residents older than 4 years in El Kolsam, Kafr Saad, and Tahoria villages. This survey was conducted as part of a longitudinal study of filariasis prevalence and incidence in these villages. We also performed a whole-village survey in 1998 of residents older than 4 years in Kafr Tahoria village. These surveys identified infected subjects who were treated with single-dose DEC and restudied 1 year after treatment in 1999 to assess effects of this treatment on MF and filarial antigenemia.
The effect of mass treatment with single-dose DEC was studied in Kafr Tahoria by performing a follow-up survey of ~25% of randomly selected households in that village in the year 2000, 27 months after treatment. This survey screened subjects with the AMRAD ICT filariasis test; subjects with positive antigen card tests had night blood collected for MF detection by membrane filtration. Our previous work in Kafr Tahoria provided a special opportunity to examine changes in filariasis prevalence rates and infection intensities in this village over time; results obtained in Kafr Tahoria in 1998 and 2000 were compared with data obtained in this village by our group in surveys conducted in 1990/12 (60% sample, age > 9 years), 199411 (10% sample, age > 9 years), and 2000 (25% sample, age > 4 years).

Informed consent was obtained from all study subjects (and from parents of minors) for participation in this study. The study was approved by institutional review boards at Ain Shams University in Cairo and at Barnes-Jewish Hospital in St. Louis, Missouri. The Egyptian MOHP also approved the study.

Specimen collection. Field teams, comprising a physician, a technician, and one or more local village residents, visited houses in the evening. After obtaining informed consent, the teams recorded demographic information on preprinted forms. Venous blood samples were collected between the hours of 9 PM and 1 AM for parasitology and serology studies. Fingerprick blood samples were collected at the same time for thick smears and for performance of the AMRAD ICT Filariasis Test.

Tests for *W. bancrofti* infection. Microfilariae were detected by microscopic examination of Giemsa-stained thick smears prepared with 50 μL of blood collected by fingerprick. Microfilariae were also detected by membrane filtration (5 μM; Nuclepore Corp., Pleasanton, CA) of 1 mL venous blood and microscopic examination of stained filters.

Filarial antigen was detected in plasma samples by enzyme-linked immunosorbent assay (ELISA), as previously described in detail.13,14 Antigen testing was also performed in the field with finger prick blood samples with the AMRAD ICT Filariasis Test (AMRAD ICT, French’s Forest, Australia) according to the manufacturer’s instructions. This test is a rapid-format immunochromatography card test.15 Card test results were read visually in the field after 15 minutes.

Diethylcarbamazine treatment. All residents of Kafr Tahoria village > 4 years old (excluding pregnant women) were offered treatment (“mass treatment”) with a single 6 mg/kg orally administered dose of diethylcarbamazine citrate (Pharmamed Ltd., Zejtun, Malta). Treatment was directly observed by study personnel. Residents of sampled populations in other villages were selectively treated with single-dose DEC only if their blood tests revealed MF (by membrane filtration) or filarial antigenemia (AMRAD ICT filariasis test).

Data analysis. Database management and statistical analyses were performed with Epi Info software,16 version 6. Proportions were compared by chi-square analysis or Fisher’s exact test (2-tailed). Changes in continuous variables after treatment were assessed with the Wilcoxon signed-rank test for repeated measures. The Mann-Whitney U-test was used to assess significance of group differences for continuous variables.

### RESULTS

**Parasitology.** Baseline (1998) MF prevalence and infection intensity were low in the study population (Table 1). This was due in part to the fact that many subjects in these villages had been treated with DEC in recent years.

Effects of single-dose DEC treatment on MF in infected subjects are summarized in Table 2. Thick smear MF results were available for 20 people who were positive at baseline in 1998 and retested in 1999, 1 year after treatment with single-dose DEC. Microfilaria counts were reduced by an average ± standard error of 85.5 ± 0.4% in these subjects, and 12 (60%) of 20 had negative smears in 1999. Microfilaria filter data were available for 29 people who were positive before treatment and retested 1 year after treatment. Microfilaria counts were reduced by 89.4 ± 4.1% in these people, and complete MF clearance was observed in 20 (69%) of 29 patients. Clearance was significantly more frequent when pretreatment MF counts were ≤ 100/mL (17 of 19 subjects) than when pretreatment counts were > 100/mL (3 of 10) ($P = 0.004$, Fisher’s exact test). The rates of MF clearance after treatment by thick smear and by membrane filtration were not significantly different by the chi-square test.

**Filarial antigen card test: baseline data and sensitivity in MF carriers.** Pretreatment filarial antigen prevalence by the card test was much higher than MF prevalence rates (Table 1). The sensitivity of the card test in subjects with MF was 59 of 70 (84.3%) versus membrane filter and 50 of 53 (94.4%) versus thick smear.

Effect of single-dose DEC on filarial antigenemia as assessed by the card test. Eighty-six people with positive antigen card tests in 1998 were retested 1 year after single-dose DEC treatment; 35 of these people had negative card tests in 1999 (clearance rate, 40.7%). Seroconversion from positive to negative was more common when pretreatment antigen levels were < 10 ng/mL (28 of 52, 53.8%) than when pretreatment levels were greater than or equal to 10 ng/mL (6 of 33, 18.1%) ($P < 0.01$). Ten (35.7%) of 28 people with positive antigen card tests and MF by membrane filtration before treatment had negative antigen card tests 1 year after treatment.

**Effect of single-dose DEC therapy on MF incidence in endemic normal subjects with filarial antigenemia by the card test.** Previous studies have shown that filarial antigen tests detect filarial antigen in a subset of endemic normal subjects (clinically normal, amicrofilaremic individuals who live in filariasis-endemic areas). These subjects are believed to have microfilaremics (reviewed in Weil and others).37

<table>
<thead>
<tr>
<th>Test</th>
<th>No. tested</th>
<th>Positive (%)</th>
<th>Range</th>
<th>Mean ± standard error</th>
<th>Median</th>
</tr>
</thead>
<tbody>
<tr>
<td>MF, smear (50 μL)†</td>
<td>1,901</td>
<td>2.8</td>
<td>1–54</td>
<td>12.7 ± 1.7</td>
<td>6.0</td>
</tr>
<tr>
<td>MF, filter (1 mL)</td>
<td>1,891</td>
<td>3.7</td>
<td>1–755</td>
<td>107 ± 18.2</td>
<td>34.0</td>
</tr>
<tr>
<td>Antigen (card)</td>
<td>1,910</td>
<td>NA</td>
<td>NA</td>
<td>148 ± 14.1</td>
<td>8.0</td>
</tr>
</tbody>
</table>

* ELISA = enzyme-linked immunosorbent assay; MF, microfilaria; NA, not applicable.
† Most subjects had all 4 tests performed. The numbers tested vary, however, because of technically inadequate or lost smears and filters in some cases and because of the lack of sufficient serum for the antigen ELISA.
‡ Values are in nanograms per milliliter.
Two of 58 endemic normal subjects with positive antigen card tests in 1998 who were retested for MF by membrane filtration had MF in 1999. This MF incidence rate (3.4%) is significantly lower than the MF incidence rate of 21% observed in 67 untreated antigen-positive endemic normal subjects in the same villages between 1994 and 1995 (P = 0.006, Fisher's exact test). This result suggests that treatment may have prevented MF incidence in antigen-positive endemic normal subjects. Twenty-five (43%) of 58 endemic normal subjects with positive antigen card tests in 1998 had negative antigen card tests in 1999. This antigen clearance rate was not significantly different from that seen in microfilaraemic subjects.

**Filarial antigen ELISA: baseline data and sensitivity in MF carriers.** Pretreatment filarial antigen prevalence by ELISA was comparable to that obtained by the card test and much higher than MF prevalence rates (Table 1). The sensitivities of antigen testing by ELISA in people with MF by membrane filtration and thick smear were 53 (84.1%) of 63 and 43 (89.6%) of 48, respectively. These values are not significantly different from sensitivities obtained with the antigen card test. ELISA and card test results were the same in 1,623 (96%) of 1,693 patients.

**Effect of single-dose DEC on filarial antigenemia as assessed by ELISA.** One advantage of ELISA over the antigen card test is its ability to quantitate filarial antigen levels in serum or plasma. Pretreatment antigen levels (a measure of infection intensity) in this population were low; the mean antigen level in those with positive tests was 14.8 ± 1.4 ng/mL before treatment (median, 9.0 ng/mL). Quantitative antigen testing was performed on the same ELISA plates with sera collected before and 1 year after treatment for those with pretreatment antigen levels greater than or equal to 10 ng/mL. Antigen levels fell by an average of 61.0 ± 4.5% after treatment, and antigenemia was completely cleared in 4 (13.3%) of 30 subjects. This rate of clearance was slightly lower than that observed by the card test for subjects with antigen levels in the same range (6 of 33, 18.1%), but this difference was not statistically significant (P = 0.85). The decrease in filarial antigen levels after treatment was greater in those with MF before treatment than in antigen-positive endemic normal subjects (reduction, 72.3 ± 5.0% versus 46.2 ± 6.1%, respectively; difference significant by Mann-Whitney U-test, P < 0.01).

**Effect of single-dose DEC therapy on MF incidence in endemic normal subjects with filarial antigenemia by ELISA.** One (3.4%) of 29 endemic normal subjects with positive ELISA tests for filarial antigen in 1998 had MF by membrane filtration in 1999. Again, this is significantly lower than the 21% MF incidence rate observed for 67 untreated antigen-positive endemic normal subjects studied in the same area in 1994–1995 (P = 0.03, Fisher's exact test).

### Table 2

Effects of single-dose diethylcarbamazine treatment on *Wuchereria bancrofti* infections in subjects retested 12 months after treatment

<table>
<thead>
<tr>
<th>Test</th>
<th>No. tested</th>
<th>Mean ± SE</th>
<th>Median</th>
<th>Pretreatment</th>
<th>Mean ± SE</th>
<th>Decline, mean ± SE</th>
<th>Total clearance (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MF, Smear (50 μL)</td>
<td>20</td>
<td>13.2 ± 2.6</td>
<td>8.0</td>
<td>3.9 ± 0.7</td>
<td>85.5 ± 0.4</td>
<td>60</td>
<td></td>
</tr>
<tr>
<td>MF, Filter (1 mL)</td>
<td>29</td>
<td>99.9 ± 26.9</td>
<td>31.5</td>
<td>57.6 ± 15.1</td>
<td>89.4 ± 4.1</td>
<td>69</td>
<td></td>
</tr>
<tr>
<td>Ag (card)</td>
<td>86</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>40.7</td>
<td></td>
</tr>
<tr>
<td>Ag (ELISA)</td>
<td>65</td>
<td>15.9 ± 2.1</td>
<td>11.0</td>
<td>11.1 ± 1.4</td>
<td>61 ± 4.5</td>
<td>13.3</td>
<td></td>
</tr>
</tbody>
</table>

*Ag = antigen, ELISA = enzyme-linked immunosorbent assay; NA = not applicable; SE = standard error. MF = microfilaremia.*

### Table 3

Changes in microfilaremia treatment and intensity in Kafr Tahoria Village, 1990–2000

<table>
<thead>
<tr>
<th>Year</th>
<th>No. tested</th>
<th>No. positive</th>
<th>% positive</th>
<th>Results in MF-positive subjects</th>
<th>Results for all people tested</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Mean ± SE</td>
<td>Mean ± SE</td>
</tr>
<tr>
<td>1990</td>
<td>532</td>
<td>145</td>
<td>27.2</td>
<td>278.7 ± 14.5</td>
<td>35.1 ± 0.8</td>
</tr>
<tr>
<td>1994</td>
<td>116</td>
<td>7</td>
<td>6.0</td>
<td>24.0 ± 1.0</td>
<td>10.8 ± 0.8</td>
</tr>
<tr>
<td>1998</td>
<td>755</td>
<td>37</td>
<td>4.9</td>
<td>100.2 ± 1.0</td>
<td>24.1 ± 0.8</td>
</tr>
<tr>
<td>2000</td>
<td>243</td>
<td>2</td>
<td>0.8</td>
<td>49.5 ± 1.0</td>
<td>40.5 ± 0.8</td>
</tr>
</tbody>
</table>

*Details of methods used in these surveys are included in the text. Subjects with microfilaraemia detected in surveys in 1990 and 1994 were treated with diethylcarbamazine (DEC), 6 mg/kg for 12 days. All people studied in 1998 were treated with a single 6 mg/kg dose of DEC. Data shown are for subjects older than 9 years. MF = microfilaremia; SE = standard error.*
Changes in prevalence* and levels† of filaria antigenemia in Kafr Tahoria Village, 1990–2000

<table>
<thead>
<tr>
<th>Year</th>
<th>No. tested</th>
<th>No. positive</th>
<th>% positive†</th>
<th>Antigen (mean ± standard error)‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>1990</td>
<td>533</td>
<td>203</td>
<td>38.1</td>
<td>41.7 ± 22.3</td>
</tr>
<tr>
<td>1994</td>
<td>116</td>
<td>15</td>
<td>12.9</td>
<td>NA</td>
</tr>
<tr>
<td>1998</td>
<td>702</td>
<td>62</td>
<td>8.8</td>
<td>NA</td>
</tr>
<tr>
<td>2000</td>
<td>268</td>
<td>35</td>
<td>13.1</td>
<td>10.6 ± 0.2</td>
</tr>
</tbody>
</table>

* ICT Card Test in 1998 and 2000; enzyme-linked immunosorbent assay (ELISA) before 1998. Data shown are for ages > 5 years.
† Filarial antigen prevalence rates in 1994, 1998, and 2000 were not significantly different by chi-square test (P = 0.13).
‡ Antigen levels were simultaneously determined by ELISA for a random sample of sera from antigen-positive subjects collected in 1990 and 2000. The difference in antigen levels is statistically significant (Mann-Whitney U-test, P < 0.01).

4 show that mean antigen levels in antigen-positive subjects in this village decreased in this interval by ~ 75%.

DISCUSSION

The purpose of this study was to assess the effect of single-dose DEC treatment of filariasis in a low-endemicity setting. Baseline infection prevalence rates and intensities (MF counts and antigen levels in 1998) were low in the study population. This is due in part to the fact that many subjects in the study area had been treated with DEC in recent years. The large disparities between antigen and MF prevalence rates (Table 1) also probably reflect previous filariasis treatment in the study area; previous studies have shown that DEC treatment can clear MF without clearing antigenemia. Persistent antigenemia suggests survival of adult worms after treatment; microfilariae are believed to be more sensitive to DEC than adult worms.

Filarial antigen tests detect adult worm products in human blood, and positive tests are believed to indicate the presence of living adult worms in the host. Previous studies have shown that the antigen-capture ELISA and the card test used in this study have comparable sensitivities, with positive tests in 90–97% of sera or plasma from untreated MF carriers. Filarial infection reduces adult worm infection intensities and antigen levels. Thus, prior treatment of many infected subjects in the study area (and unusually low filarial antigen levels in the study population) may explain the relatively low sensitivity of antigen testing in microfilaremic subjects observed in this study. Similar results have been reported in previous studies of sera from low-endemicity areas in the South Pacific after mass treatment.

Single-dose DEC had a dramatic effect on MF levels, with MF clearance at 12 months in a high percentage of subjects. Treatment with DEC also seems to have prevented MF incidence in antigen-positive endemic normal patients; this inference is based on a comparison of the low MF incidence rate observed in this study with the high rate observed in untreated antigen-positive endemic normal patients in the same villages in 1994–1995. This is an important advantage of mass treatment over the traditional practice of selective treatment of MF carriers.

Effects of single-dose DEC on filarial antigenemia were also significant, but less dramatic. Previous studies have shown that filarial antigen levels decrease by ~ 50% after single-dose DEC treatment, and a 61% decrease was observed in the present study. Previous studies have also reported that filarial antigen tests usually remain positive after single-dose DEC treatment, and this was also true for the majority of subjects in the present study, including some who completely cleared MF after treatment. However, the observation that the antigen card test converted to negative in some subjects after treatment (especially in those with low pretreatment antigen levels) suggests that this test may be useful for monitoring effects of mass treatment programs, especially after multiple treatment cycles.

The longitudinal results from the village of Kafr Tahoria are especially interesting. This village had one of the highest filariasis prevalence rates measured by the Egyptian MOHP before we initiated our studies there in 1990. Although slightly different sampling methods were used at different times (as described above), it is clear that both the prevalence and intensity of filariasis infection decreased dramatically in this village between 1990 and 2000. The decline before 1998 can be attributed in part to selective treatment of MF carriers with (then) standard 12-day courses of DEC. In addition, filariasis also declined without extensive treatment in other areas in Egypt during this time for reasons that are currently not understood. However, mass distribution of single-dose DEC in 1998 had dramatic and durable effects on MF prevalence in Kafr Tahoria.

In contrast to the decrease in MF prevalence, filarial antigen prevalence did not decline in Kafr Tahoria between 1998 and 2000. Obviously, filarial antigen testing does not accurately reflect the effect of mass treatment programs on MF prevalence rates. The significance of persistent filarial antigenemia in this village 27 months after mass treatment is unclear at present. It is likely that this indicates survival of adult worms in many treated subjects, despite their frequent clearance of MF. If this is true, persistent filarial antigenemia in areas after mass treatment may signify that these areas are at risk for later recrudescence of filariasis if mass treatment is prematurely discontinued. This hypothesis needs to be tested, but it will be difficult to do this in Kafr Tahoria, because this village was mass treated with DEC and albendazole in 2000 and 2001 as part of the Egyptian MOHP’s National Filariasis Elimination Program.

Our study has other potentially important implications for the growing Global Programme for Elimination of Lymphatic Filariasis. The study was performed in a low-prevalence area with light infection intensities; many of our subjects had been previously treated with standard courses of DEC by our group or the Egyptian MOHP. This adds real-world relevance to our results, because these conditions also apply in many other endemic areas around the world. In addition, areas that are highly endemic now are likely to resemble our study area after 1–2 cycles of mass treatment with DEC and albendazole. Although the World Health Organization has advocated repeated, annual mass treatment with drug combinations as a strategy for elimination of lymphatic filariasis, mass treatment with DEC alone may be sufficient to eliminate the infection in carefully selected areas. Treatment with DEC monotherapy might also be a reasonable option for later phases of elimination programs, after infection prevalence rates and intensities have been reduced by 1–2 cycles of combination therapy.

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