# *Wuchereria bancrofti* microfilariae and quantitative circulating antigen detection in selected endemic areas in Egypt

Iman R. Abdel-Shafi[a], Eman Y. Shoeib[a], Samar S. Attia[a], José M. Rubio[b], Ayman A. El-Badry[a]

[a]Medical Parasitology Department, Kasr Al Ainy Faculty of Medicine, Cairo University, Cairo, Egypt, [b]Malaria and Emerging Parasitic Diseases Laboratory (MAPELab), National Microbiology Center, Instituto De Salud Carlos III, Madrid, Spain

Correspondence to Iman R. Abdel-Shafi, MD, Medical Parasitology Department, Kasr Al Ainy Faculty of Medicine, Cairo University, El-Manial, PO Box 11562, Cairo, Egypt
Tel/fax: +20 223 632 733;
e-mail: irriad@kasralainy.edu.eg

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**Back ground and objective**

*Wuchereria bancrofti* is responsible for 90% of cases of lymphatic filariasis throughout the tropics and in some subtropical areas worldwide, including Egypt. To combat this disease, the WHO has launched a program aiming to eliminate lymphatic filariasis by the year 2020 in all the endemic countries using mass drug administration (MDA) to interrupt the disease's transmission. The aim of the present work was to study *W. bancrofti* infection in selected endemic areas in Egypt by performing parasitological examination and enzyme-linked immunosorbent assay (ELISA) antigen detection test, and to analyze the demographic, clinical, and MDA data of the study population in relation to *W. bancrofti* infection.

**Patients and methods**

A total of 300 blood samples were collected from residents in endemic areas in five governorates. Parasitological examination and Og4C3 ELISA test were performed to identify *W. bancrofti* infection.

**Results**

Microfilariae were identified in one individual while circulating filarial antigens (CFAs) were detected in 10 individuals. Statistical analysis of the collected data showed that CFAs were significantly higher in the male population than in the female population, whereas analysis regarding other demographic, clinical, and MDA data showed no statistical significance.

**Conclusion**

The study results showed that CFAs are still detected in endemic communities in Egypt, and that the prevalence is higher in the male population than in the female population. Although the Og4C3 ELISA test is a useful research tool for the study of *W. bancrofti* infections, its cost and format hinder its wide use in endemic areas.

**Keywords:**

circulating filarial antigens, Egypt, Og4C3 enzyme-linked immunosorbent assay, *Wuchereria bancrofti*

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## Introduction

Lymphatic filariasis (LF) is a major vector-borne health problem affecting more than 120 million people in over 80 developing endemic countries. *Wuchereria bancrofti*, which is responsible for 90% of cases, is focally endemic in Egypt [1,2].

To combat this disease, the WHO has launched a 'Global Program to Eliminate Lymphatic Filariasis' (GPELF), aiming to eliminate LF by the year 2020 in all the endemic countries using mass drug administration (MDA) to interrupt the disease's transmission. Egypt was one of the first countries to implement a national program to eliminate LF based on WHO's strategy of repeated rounds of annual treatment in the form of albendazole with diethylcarbamazine (DEC) [3,4].

The exponential growth of GPELF has highlighted the need for sensitive tools to monitor progress toward programmatic endpoints, as well as aid in rapid and early detection of cases, which is often challenging [5].

LF is characterized by a wide range of clinical presentations. One group of individuals in the endemic community shows no clinical manifestations or microfilariae (Mf), which may be due to insufficient exposure, prepatent infection or adult worm infection without Mf, or clearance of infection. Another group of individuals in the endemic community shows Mf in their blood without obvious clinical manifestations. Some of these may remain microfilaremic and asymptomatic for years or even for the rest of their lives, whereas the rest may become symptomatic, with a devastating outcome [6,7].



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As regards the laboratory diagnosis of LF, the night-time blood collection for Mf detection and the low prevalence of parasites can be problematic. These obstacles in the direct methods for parasite detection have led to the development of alternative methods for diagnosis, such as immunological assays. In fact, diagnosis of bancroftian filariasis has been revolutionized by the introduction of circulating filarial antigen (CFA) tests. Although several groups have reported the detection of soluble CFAs in human blood through different immunological techniques, only two assays are currently available: an enzyme-linked immunosorbent assay (ELISA) and an immunochromatographic test (ICT) rapid test [8–12].

## Objective

The aim of the present work was to study *W. bancrofti* infection in selected endemic areas of Egypt by performing parasitological examination for Mf detection and the Og4C3 ELISA test, a marker of adult worm burden. The study also aimed to analyze the demographic, clinical, and MDA data of the study population in relation to *W. bancrofti* infection.

## Patients and methods

The study setting and most of the sampling in the present work were finalized under the authorization and guidance of the Egyptian Ministry of Health and Population (MOHP) – Malaria, Filariasis and Leishmaniasis Control Department, between October 2012 and March 2014.

### Study population

The study participants were residents in filariasis endemic areas and included normal endemic individuals, chronic cases, acute or recently diagnosed cases, and patients suspected to have LF referred to the Diagnostic and Research Unit of Parasitic Diseases, Medical Parasitology Department, Cairo University.

### Ethical considerations

The study sampling and laboratory procedures were reviewed and approved by the Research Ethics Committee of the Faculty of Medicine, Cairo University (N-42-2013). Informed consent was obtained from each participant.

### Study sampling

A total of 300 blood samples were collected from residents in filariasis endemic areas in five governorates: Qualioubiya (Kafr Ammar, Barshoum, and El Salheya villages), Menoufiya (Dalhamo village), Dakahliya, Giza (Bani Salama village), and Sharkiya governorate. All blood samples were collected in the daytime except for those collected from residents in Qualioubiya and part of Giza, which were collected at night. Relevant demographic, clinical, and MDA data were obtained with each blood sample.

### Parasitological examination

Thick blood films were prepared for the blood samples collected at night. As a part of their routine work, MOHP personnel prepared the thick blood films, examined them in their laboratory, and reported the results. Knott's concentration technique was adopted for the blood samples collected in the daytime, as followed by Garcia [13], by thoroughly mixing 1 ml of whole blood in 10 ml of 2% formalin and centrifuging for 5 min at 300*g*. The supernatant was decanted and thick films were prepared from the remaining sediment and stained with Giemsa for microscopic examination.

### Enzyme-linked immunosorbent assay antigen detection test

A volume of 100 µl of plasma was prepared from each blood sample, which was subjected to the ELISA test for the detection of *W. bancrofti* antigens using a TropBio ELISA Kit (Cellabs Pty Ltd, Sydney, NSW, Australia) following the manufacturer's instructions. Plasma samples were boiled in an EDTA solution and centrifuged. This treatment was carried out according to the manufacturer's instructions to dissociate antigen/antibody complexes, thus retaining the heat stable target antigen in the supernatant. The 96-well microtiter plates supplied were coated with a monoclonal antibody (Og4C3), which has been shown to specifically recognize only *W. bancrofti* antigens in human sera. The plate was read using the DYNEX MRX plate reader (Dynatec Laboratories, Chantilly, VA, USA) at a dual wavelength of 450/630 nm and was blanked against air. For the test results to be accepted, standard 1 (S1), which is the negative control as it does not contain any antigens, must read an optical density below 0.2 and standard 7 (S7), which is the positive control, must read an optical density higher than 2.0. Using the seven standards, it was possible to allocate the test samples into titer groups; group 1 samples were considered nonreactors, group 2 were equivocal reactors, and titer groups 3–7 were positive reactors containing CFAs.

### Statistical analyses

Data were analyzed using IBM statistical package for the social sciences (SPSS) advanced statistics (version 22; SPSS Inc., Chicago, Illinois,

USA). Numerical data were expressed as mean, SD, and range. Qualitative data were expressed as frequency and percentage. The $\chi^2$-test or Fisher's exact test was used to examine the relation between qualitative variables. For quantitative data, comparison between two groups was done using either the Student *t*-test or the Mann–Whitney test (nonparametric *t*-test) as appropriate.

## Results

On the whole, the study population (total = 300) comprised 57.7% female and 42.3% male individuals, with ages ranging from 9 to 86 years (mean = 32.6 years). The study population included endemic normal individuals (91.7%), clinically suspected cases presenting with lower-limb edema (3%), asymptomatic LF cases diagnosed during screening performed by the MOHP personnel (1%), and chronic LF cases suffering from chronic lymphoedema or hydrocele (4.3%). Overall, 10.4% of the study population had a household member diagnosed or suffering from LF, and 71.7% gave a history of MDA intake.

As regards the thick blood films, out of 95 blood films prepared from blood samples collected at night, Mf was detected in one sample only, which was considered the reference sample in the present study. No Mf was detected using Knott's technique in the 205 blood samples collected during daytime.

Out of 300 blood samples, *W. bancrofti* CFAs were detected in 10 (3.3%) samples, including the reference sample. These samples were allocated into titer groups in relation to their antigen level, with the reference sample showing the highest antigen level in titer group 6 (Fig. 1). Two samples were found equivocal even after repetition of the test; they were considered as negative samples. The rest of the samples were negative. Out of the 10 positive CFA samples, seven samples were collected at daytime and three samples at night. With regard to residence, nine participants were residing in Giza and one in Menoufiya.

Our results revealed that two female and eight male participants were positive for CFAs (Table 1). CFA-positive cases ranged in age from 22 to 44 years, with a mean age of 31.6 years (Fig. 2). Nine CFA-positive cases were endemic normal individuals and one case was asymptomatic (Table 2). Only one CFA-positive case gave a history of having a household member suffering from LF (Table 3). Moreover, eight CFA-positive cases gave a history of MDA intake (Table 4).

## Discussion

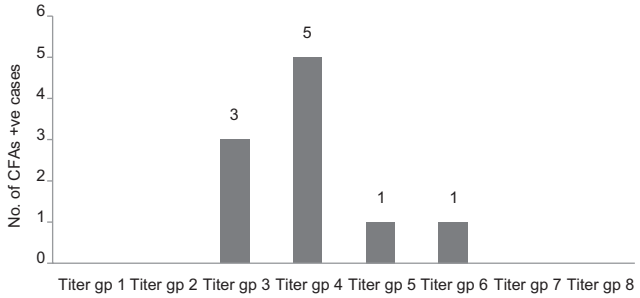
The introduction of CFA tests, the Og4C3 ELISA and the AD-12 ICT, is a major breakthrough for mapping LF and overcomes the many problems associated with the parasitological detection of Mf. Although the ICT was the preferred method by the GPELF for field application in resource-limited settings, both CFA tests showed high sensitivity and specificity. Furthermore, they enabled the detection of microfilaremic and amicrofilaremic infections from samples collected at any time of the day [14–16].

**Table 1 Distribution of positive cases in relation to sex**

| | ELISA | | Total |
|---|---|---|---|
| | Negative | Positive | |
| Sex | | | |
| Male | | | |
| Count | 119 | 8 | 127 |
| % within sex | 93.7 | 6.3 | 100.0 |
| Female | | | |
| Count | 171 | 2 | 173 |
| % within sex | 98.8 | 1.2 | 100.0 |
| Total | | | |
| Count | 290 | 10 | 300 |
| % within sex | 96.6 | 3.4 | 100.0 |

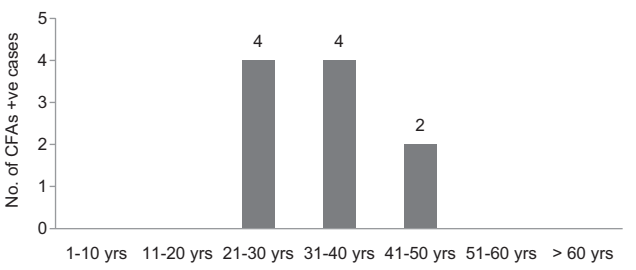
ELISA, enzyme-linked immunosorbent assay. The relation is significant as $P<0.05$ ($P=0.014$).

**Figure 1**




Distribution of circulating filarial antigen (CFA)-positive cases into titer groups (total = 10).

**Figure 2**




Distribution of circulating filarial antigen (CFA)-positive cases as per age group (total = 10).

**Table 2 Distribution of positive cases in relation to lymphatic filariasis infection status**

| | ELISA | | Total |
|---|---|---|---|
| | Negative | Positive | |
| Clinical status | | | |
| Endemic normal | | | |
| Count | 266 | 9 | 275 |
| % within clinical status | 96.7 | 3.3 | 100.0 |
| Suspected cases | | | |
| Count | 9 | 0 | 9 |
| % within clinical status | 100.0 | 0.0 | 100.0 |
| Asymptomatic cases | | | |
| Count | 2 | 1 | 3 |
| % within clinical status | 66.7 | 33.3 | 100.0 |
| Chronic cases | | | |
| Count | 13 | 0 | 13 |
| % within clinical status | 100.0 | 0.0 | 100.0 |
| Total | | | |
| Count | 290 | 10 | 300 |
| % within clinical status | 96.6 | 3.4 | 100.0 |

ELISA, enzyme-linked immunosorbent assay. No *P* value because of small number of cases within subgroups.

**Table 3 Distribution of positive cases in relation to the lymphatic filariasis status of the household members**

| | ELISA | | Total |
|---|---|---|---|
| | Negative | Positive | |
| Household members | | | |
| No | | | |
| Count | 260 | 9 | 269 |
| % within household members | 96.6 | 3.4 | 100.0 |
| Yes | | | |
| Count | 30 | 1 | 31 |
| % within household members | 96.8 | 3.2 | 100.0 |
| Total | | | |
| Count | 290 | 10 | 300 |
| % within household members | 96.6 | 3.4 | 100.0 |

ELISA, enzyme-linked immunosorbent assay. The relation is nonsignificant as $P>0.05$.

**Table 4 Distribution of positive cases in relation to mass drug administration intake**

| | ELISA | | Total |
|---|---|---|---|
| | Negative | Positive | |
| MDA received | | | |
| No | | | |
| Count | 85 | 2 | 87 |
| % within MDA received | 97.7 | 2.3 | 100.0 |
| Yes | | | |
| Count | 205 | 8 | 213 |
| % within MDA received | 96.2 | 3.8 | 100.0 |
| Total | | | |
| Count | 290 | 10 | 300 |
| % within MDA received | 96.6 | 3.4 | 100.0 |

ELISA, enzyme-linked immunosorbent assay; MDA, mass drug administration. The relation is nonsignificant as $P>0.05$.

Ramzy *et al.* [17] evaluated the impact of a single dose of DEC on *W. bancrofti* infections in Egypt. The baseline data demonstrated *W. bancrofti* infection in 2.8, 3.7, and 9.2% of the study population by using thick blood films, membrane filtration, and CFAs tests, respectively. The sensitivity of CFA testing by ELISA in people with Mf by means of membrane filtration and thick smear was 84.1 and 89.6%, respectively.

Higher sensitivity was demonstrated by Nuchprayoon *et al.* [18] in Thailand. They compared Og4C3 ELISA and ICT tests; both CFA assays could detect all microfilaremics. The Og4C3 ELISA detected 14.8% of amicrofilaremics, whereas the ICT test identified 8.1%. Similarly, Rocha *et al.* [19] in Brazil reported that the results of the Og4C3 ELISA and ICT concurred 100% and identified 96.69% of the Mf-positive individuals.

In another study in Brazil, Oliveira *et al.* [20] evaluated parasitological and CFAs tests for the detection of *W. bancrofti* infection. CFA prevalence was markedly higher than Mf prevalence. The sensitivity of the Og4C3 ELISA test was ~100%, measured against a combination of parasitological tests: thick blood film, Knott's technique, and membrane filtration. The estimated specificity of the Og4C3 ELISA was higher than that of the ICT.

Although the Og4C3 ELISA is considered a marker of adult worm burden, it is partially associated with Mf as well. Early studies have shown that the sensitivity of the Og4C3 test was absolute (100%) for patients with an Mf density more than 30 Mf/ml but fair (<75%) for the low Mf carriers, suggesting the limitations of the test when detecting infections with low parasite burdens [21,22]. In the same context, Rocha *et al.* [19] highlighted the concern that currently available diagnostic tools for LF may have severe limitations when parasite prevalence and antigen levels are low.

Likewise, Gass *et al.* [23] described a multicenter study to evaluate diagnostic tools for GPELF. They found that the overall levels of positivity were similar for the CFA tests: ~9% were positive by ICT and 8% by Og4C3. However, at the individual level the tests differed significantly. Statistical comparison of the results found the two CFA tests to be significantly different. In addition, Oliveira *et al.* [20] highlighted the need for better standardization of the Og4C3 ELISA technique and better quality control of the kits by the manufacturer, particularly with regard to the plates and reagents.

Concerning the demographic characteristics of the positive cases in the present study, the CFA-positive cases included eight male and two female individuals, with a statistically significant relation between them (Table 1). Likewise, various studies using different diagnostic tools have demonstrated higher rates of infection in the male population [24–26].

However, in the study by Weil *et al.* [27] on *W. bancrofti* infection in the Nile delta, no significant sex differences were observed in filarial antigen, Mf, or antibody prevalence rates. Further, Nuchprayoon *et al.* [18] using the Og4C3 test found no statistically significant sex-related differences in the prevalence of LF in Thailand.

In the present study, the CFA-positive cases were allocated into three age groups, ranging from 20 to 50 years (Fig. 2). Similarly, in a study performed in Egypt the prevalence rates for LF disease, microfilaremia, antigenemia, and antifilarial antibodies were found to be significantly lower in children aged 10–19 years than in older individuals [27].

Simonsen *et al.* [28] highlighted the role of transmission intensity as an important determinant of observed intercommunity variation in infection, disease, and host response patterns in bancroftian filariasis as they reported that both infection and disease appeared earlier and reached much higher levels in high-endemic than in low-endemic communities. They observed that the overall and age-specific infection and disease patterns in the two communities were in agreement with the view that they are primarily shaped by transmission intensity.

The current study results revealed that the majority of positive cases (90%) were endemic normal (Table 2). This demonstrates asymptomatic infection, in which the individual does not have any evidence of clinical filarial disease while harboring living parasites, adult with or without Mf. Usually diagnostic tests are positive at this stage. Once irreversible chronic lymphatic pathology is established, generally after treatment or death of the filarial parasite, the diagnostic tests become negative [29–31]. However, it is worth noting that CFAs tests may yield positive results after treatment even when there is no viable infection [15,20].

The study data showed that only one CFA-positive case reported having a household member diagnosed with LF, and the relation was considered nonsignificant (Table 3). In agreement with the present finding, Leite *et al.* [32] in a study aiming to evaluate the occurrence of LF infection using different diagnostic tests revealed no infected individuals in the family of the microfilaremic individual. Nevertheless, some studies have indicated that household contacts with microfilaremic individuals have a greater chance of acquiring the infection than does the general population [32]. The risk associated with proximity to infected persons becomes of particular interest as communities see fewer and fewer instances of new infections. Washington *et al.* [33] and Drexler *et al.* [34] observed substantial risk with spatial proximity to a CFA-positive person in terms of both exposure as well as acquisition of LF, arguing that clustering may play a substantial role in transmission dynamics.

In the current study, positive cases were found in two governorates, Giza (9%) and Menoufiya (1%). Overall, 80% of positive cases gave a history of MDA intake, without statistical significance (Table 4). It is worth noting that DEC kills Mf and a proportion of adult worms. It was suggested that DEC modifies Mf and they are cleared by the host immune system; blood Mf counts decrease quickly after treatment but tend to reappear a few months later. Therefore, MDA is carried out 4–6 times yearly, which corresponds to the average fecundity of the adult worms. Albendazole has some activity against adult parasites, causing a slow decrease in Mf counts over a period of months [35,7].

In Egypt, various studies were performed to evaluate the effect of MDA in several endemic areas. The largest study to date was conducted by Ramzy *et al.* [36], which described changes in Mf and Og4C3 antigen after five annual rounds of MDA in four sentinel villages in Giza and Qualioubiya governorates. The study results showed reduction in the Mf reservoir such that the currently accepted elimination threshold of 1% prevalence was achieved in Qualioubiya, where no Mf-positive individuals were detected by thick blood smear, and reached 1.2% in Giza. With respect to CFAs, it was shown to be decreased to a great extent.

Similarly, El-Setouhy *et al.* [37] investigated compliance with MDA in a village in Giza and reported an excellent compliance rate (>85%). However, individual compliance was highly variable: 7.4% of those surveyed after five rounds of MDA denied having ever taken the medications and 52.4% reported that they had taken all five doses. The Mf and CFA rates were 0.2 and 2.7%, respectively, in those who reported taking five doses of MDA and 8.3 and 13.8% in those who reported taking no dose. There was no significant difference in residual infection rates among those who had taken two or more doses. Furthermore, a trend toward reduced infection rates in noncompliant people was observed after several rounds of MDA, as this antifilarial medication indirectly benefits noncompliant people by a herd treatment effect [37].

However, Burkot *et al.* [38] indicated that five years of MDA with DEC and albendazole in Egypt had not been sufficient to eliminate LF transmission in areas that had high baseline infection rates. Abdel-Hamid *et al.* [39,40] reported the presence of LF cases in endemic areas in Sharkiya and Menoufiya governorates despite the fact that the collected samples were limited

and that the governorate was covered by the MDA national program of MOHP.

A systematic review of LF elimination strategies between 1980 and 2012 found that all the studies indicated significant reduction using one drug and a slightly increased benefit on using two different drugs, with a risk for recurring infection unless the final rate is less than 1%. In addition, the studies highlighted the continuous need for large conclusive research to fill this existing gap in knowledge regarding the efficacy of drug regimens [41].

## Conclusion

The study results showed that CFAs are still detected in endemic communities in Egypt, and that the prevalence is higher in the male population than in the female population. The Og4C3 ELISA test is a useful research tool for the study of *W. bancrofti* infections; however, its cost and format hinder its wide use in endemic areas.

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Nil.

### Conflicts of interest

There are no conflicts of interest.

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