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A New Case of Bancroftian Filariasis after Years since Validation of Successful Elimination in Egypt

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Abstract:

Background: Lymphatic filariasis (LF) is a mosquito-borne neglected tropical disease found worldwide in tropical and subtropical regions. Wuchereria bancrofti is responsible for more than 90% of infections. This disease can lead to significant morbidity, disability, and economic burden. According to WHO-recommended strategies, Egypt launched a national LF elimination program in 2000 and successfully achieved the elimination of LF in 2017. This study aimed to evaluate the LF transmission after years of the validation of elimination in Alkorain village, Sharqia governorate, Egypt. **Methods:** A cross-sectional study was conducted in Alkorain village, Sharqia governorate, Egypt, including patients attending the Alkorain Endemic Diseases Centre who were randomly selected at the study period and were tested for filarial antibodies; then test-positive cases underwent a provocation test followed by preparation of Giemsa-stained thick blood films. **Results:** Of 360 study participants, only one LF case (0.3%) was detected, who was a 15-year-old female student that presented with mild fever, malaise, and right lower limb swelling. Physical examination revealed pitting edema of her right leg, with several insect bites on the skin of both legs. Filariasis IgG/IgM rapid test, filarial antigen ELISA assay and stained thick blood smears indicated infection with bancroftian filariasis. The patient responded well to oral administration of a single dose of diethylcarbamazine plus albendazole. **Conclusion:** Our study concluded that the elimination of LF in Alkorain village is still maintained. Based on our case, it is necessary to consider LF in any patient from a previously endemic region who presents with unexplained limb swelling.

Keywords: Lymphatic filariasis; Wuchereria bancrofti; Limb swelling; Egyptian elimination.

Introduction

Lymphatic filariasis (LF) is considered one of the neglected tropical diseases transmitted by the infective bite of mosquitoes of the genus *Culex*, *Anopheles*, *Aedes*, and *Mansonia*. The causative parasites are three filarial nematodes: *Wuchereria bancrofti* (*W. bancrofti)*, *Brugia malayi*, and *Brugia timori*. However, *W. bancrofti* is responsible for the majority of human infections in which man is the only definitive host [1-3]. The infective third stage larvae, which are injected during mosquito bite, pass to blood circulation and then develop into adults. Adult parasites inhabit the lymphatic system while their progeny (microfilaria) pass to the blood circulation [4]. This disease can lead to prolonged suffering, significant morbidity, social stigma, and a substantial economic burden on both individuals and communities. In 2021, WHO estimated that over 882 million individuals in 44 countries globally would remain at risk of LF [5]. Despite the possibility of infection at any age, children closer to adulthood are at a greater risk of infection [6]. The risk of disease transmission may increase due to poor sanitation, urbanization, and mismanagement of waste disposal. The estimated incubation period of LF is about 8 - 26 months and could extend even longer in areas of endemicity. Although the infection may be transmitted during childhood, apparent clinical manifestations usually don't present until adolescence or adulthood. LF disease may be asymptomatic or symptomatic, with a wide range of various clinical manifestations. Besides, about 70% of infected individuals can present as asymptomatic carriers [4-8]. Acute clinical features of bancroftian filariasis include episodic attacks of lymphadenitis, lymphangitis, and lymphedema, accompanied by fever, headache, and malaise, while the chronic phase mostly manifests as hydrocele, lymphoedema, and elephantiasis [9]. People who reside in or come from areas where LF is endemic should be suspected of harboring the infection, especially those with considerable history of insect bites. However, sure diagnosis is based on the detection of the parasite itself, its antibody/antigen, or its DNA [3]. Additionally, positive results of antifilarial antibody assays serve as the earliest evidence of infection, while the lack of any filarial antibody responses may indicate the interruption of transmission [10].

Diethylcarbamazine (DEC), ivermectin, albendazole, and doxycycline are the recommended effective drugs for treatment of *W. bancrofti* and *Brugia* species infections. The combined therapy consisting of any two of these drugs can exhibit a greater efficacy in microfilarial clearance than monotherapy [3, 11]. In 2000, WHO launched the Global Programme to Eliminate LF (GPELF) based on the implementation of mass drug administration (MDA), vector control measures, management of morbidity, and prevention of disability. By 2018, there was a 74% decline in LF prevalence since the start of WHO's GPELF in 2000 [5, 12]. According to WHO-recommended strategies, Egypt launched a national LF elimination programme (NLFEP) in 2000 and successfully achieved the elimination of LF in 2017 [13].

This study was conducted to evaluate the BF transmission after years of the validation of elimination in Alkorain village, Sharqia governorate, Egypt. We report a recently discovered rare case of bancroftian filariasis in a 15-year-old Egyptian female presenting with right lower limb swelling, after many years of the national elimination programme.

Patients and Methods:

A cross-sectional study was performed from the first of August to the thirty-one of December 2022, at Alkorain village, Sharqia governorate, Egypt, to evaluate the LF transmission after years of the

validation of elimination among residents of this village. 360 individuals with an age range (5-63 years) were randomly selected from those who attended the Alkorain Endemic Diseases Centre during the period of the study and fulfilled the inclusion criteria (individuals aged more than two years old and resided in Alkorain village during the past 2 years). The study-excluded individuals were those who were less than 2 years old, didn't reside at Alkorain village during the past 2 years, or children whose legal parents refused study participation. After obtaining a verbal consent, they were tested for circulating filarial antibodies using Biopanda filariasis IgG/IgM rapid test cassette (Biopanda Reagents Ltd, United Kingdom) according to manufacture instructions. The diagnosis was confirmed by detection of specific AD12 filarial antigen using ELISA assay. The test-positive cases underwent a provocation test followed by preparation of Giemsa-stained thick blood films through direct smear and Knott's concentration procedures. Finally, these blood films were examined microscopically for detection of *W. bancrofti* microfilariae (MFs). The collected samples were processed and examined at the medical parasitology department, faculty of medicine, Benha university. Adult mosquito collection was performed in the houses of positive case by using pyrethrum spray sheet collection. The agreement of the house owner was obtained to open his house for collection, and he was free to decline the collection at any time. The collected mosquitoes were identified according to a recommended key [14-16]. The feeding and gravidity status of female mosquitoes was classified according to abdominal appearance [17].

Ethical consideration: The ethical approval was obtained from the Research Ethics Committee of Benha Faculty of Medicine (MD 18-7-2022). Examination of participants was conducted after explaining the study aim and ensuring data confidentiality; then, informed written consent was obtained from every study participant. For children, consents were obtained from their parents or legal guardians.

Statistical analysis: The data were analyzed using the Statistical Package for the Social Sciences" SPSS 22.0 software (IBM Microsoft). Kolmogorov-Smirnov test was used to test quantitative data normality of distribution. Qualitative data were described using number and percent. Quantitative data were described using range (minimum and maximum), mean, standard deviation. Since one positive case was detected, the descriptive statistics were used to summarize findings.

Results:

In the present study, the mean age of the study-included individuals was 19.86 ± 14.38 years, with a wide range of ages (5-63 years). 360 individuals were included in this study, the greatest percentage of them were children (56.9%), females (60.8%), and students (64.5%). Most of them mentioned having well-constructed houses, using anti-mosquito preventive measures (98.3%), and the availability of sanitation facilities (100%). While the presence of water collections nearby houses was mentioned by 50% of participants. The prevalence of LF infection among all examined individuals was 0.3% (one case was detected of 360 participants) **(Table 1)**.

The identified case was a 15-year-old female student who presented with mild fever, malaise, and right lower limb swelling **(Figure 1)** for four days. Physical examination revealed pitting edema of the right leg, with several insect bites on the skin of both legs. There was a history of a similar episode a few months ago, which was diagnosed as cellulitis and consequently treated with antibiotics and anti-inflammatory drugs. There was no history of any cardiac or endocrinal diseases. Doppler ultrasonography of the right lower limb veins indicated normal

deep and superficial systems. LF was suspected, and the patient was tested for circulating filarial antibodies using a Biopanda filariasis IgG/IgM rapid test cassette (Biopanda Reagents Ltd., United Kingdom). She was positive for antifilarial IgG **(Figure 2)**. The diagnosis was confirmed by detection of specific AD12 filarial antigen using ELISA assay. Then a blood sample was taken after execution of provocation test, and direct thick blood smears stained with Giemsa were negative. However, after application of Knott's concentration procedure, the MFs could be identified in stained blood smears **(Figure 3)**. Subsequently, she received a single oral dose of a combination of DEC (6 mg/kg) and albendazole (400 mg), and this combined therapy was prescribed as a single dose per year for four years. The identified case mentioned the presence of water collections nearby the house, non-usage of any anti-mosquito preventive measures, and the availability of sanitation facilities including safe water supply.

Identification of adult mosquitoes collected from the house of the positive case revealed that only *Culex pipiens* species could be detected indoors. Out of 328 collected mosquitoes, the highest proportion were females (77.7%), while males represented only 22.3%. The highest percentage of female mosquitoes were freshly fed (41.2%), followed by half gravid (26.7%), full gravid (18.4%), and unfed (13.7%) females **(Table 2)**.

Finally, the patient was followed up for two years and exhibited no recurrence of clinical symptoms of LF. Following two doses of DEC, both the Giemsa-stained thick blood smear examination and the AD12 filarial antigen ELISA test gave negative results.

Table (1): Comparison between the infected and non-infected individuals regarding personal and sociodemographic factors.

Variable		Infected		Non-infected		Total	
		n	**%**	**n**	**%**	**n**	**%**
Age	**≤ 15 years**	1	0.5%	204	99.5%	205	56.9%
	>15 years	0	0%	155	100%	155	43.1%
Sex	**Male**	0	0%	141	100%	141	39.2%
	Female	1	0.5%	218	99.5%	219	60.8%
Occupation	**Student**	1	0.4%	231	99.6%	232	64.5%
	Employed	0	0%	66	100%	66	18.3%
	Housewives	0	0%	62	100%	62	17.2%
The presence of water collections close to the house	**Yes**	1	0.6%	179	99.4%	180	50%
	No	0	0%	180	100%	180	50%
Using anti-mosquito preventive measures	**Yes**	0	0%	354	100%	354	98.3%
	No	1	16.7%	5	83.3%	6	1.7%
Total		**1**	**0.3%**	**359**	**99.7**	**360**	**100%**

Number (n).

Table (2): Adults *Culex pipiens* characteristics collected from the positive case house.

Studied Variables		n	%
Sex	**Female**	255	77.7%
	Male	73	22.3%
Total		328	100%
Feeding and gravidity of females	**Unfed**	35	13.7%
	Freshly fed	105	41.2%
	Half gravid	68	26.7%
	Full gravid	47	18.4%
Total		255	100%

Number (n).

Figure (1): A clinical photograph of the patient showing swelling of the right leg.

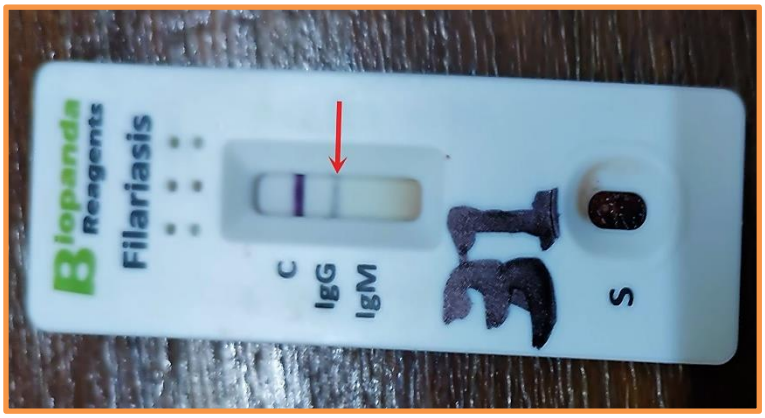

Figure (2): Biopanda filariasis IgG/IgM rapid test cassette showing positive IgG result (red arrow).

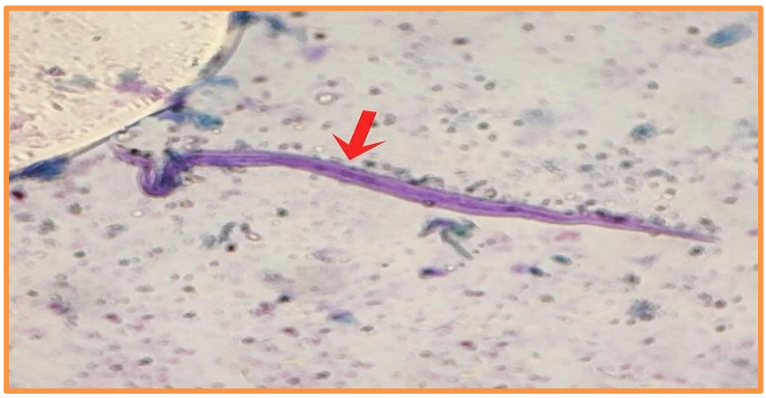

Figure (3): Giemsa stained thick blood smear showing *W. bancrofti* microfilaria (red arrow) (400x).

Discussion:

LF is a mosquito-borne disease found worldwide in tropical and subtropical regions, including West and Central Africa, Southeast Asia, the Eastern Mediterranean, the Western Pacific, Papua New Guinea, and South America. In various areas, males may exhibit higher infection rates with LF than females [11, 18].

The risk of LF infection is closely related to low socioeconomic standards, rural life, inadequate sanitation facilities, and water-logged environments. Consequently, people in poor rural areas may be more vulnerable to infectious mosquito bites. Fortunately, acquiring the infection typically requires a large number of infectious bites over several months [4, 19]. Alkorain village, located in Sharqia governorate, Egypt, was previously endemic to LF and had many cases of elephantiasis [20].

In the present study, the mean age of the study-included individuals was 19.86 ± 14.38 years, with a wide range of ages (5-63 years). The highest percentage of study participants (56.9%) were children. Doubtless, in regions without any proof of filarial infection for several years, a broader range of ages should be surveyed, especially children. The wide range may allow the analysis of age-related factors in the disease's prevalence and severity [21].

Of this study participants, the proportion of females (60.8%) was higher than males (39.2%), which revealed a higher rate of health-seeking behavior and attendance at the local endemic diseases center among females. Likewise, Yount et al.[22] reported that women had higher rates of utilization of health center services and medications compared to men in Egypt and Tunisia. Additionally, in an Egyptian village, Sameh et al.[23] found that the percentage of formal health care-seeking behavior was higher in females (50.3%) than males (49.7%).

Our study respondents were predominantly students (64.5%), so the proximity of the Alkorain endemic diseases centre to many schools can enhance students' access and utilization of health services, which may explain the higher proportion of students in our study. In this context, Yaro et al.[24] demonstrated that optimum location of educational institutions can enhance health service utilization among students.

Our findings revealed widespread of well-constructed houses, utilization of mosquito preventive measures (98.3%), and the availability of sanitary facilities (100%). There is no doubt that improved housing, the availability of sanitation facilities and a clean safe water supply, and using anti-mosquito preventive measures can significantly reduce the human-vector contact and can help in the reduction of mosquito breeding habitats and limit LF transmission potential [25, 26].

In the present study, only one case of LF (0.3%) was detected among all examined individuals. This result meets the WHO's minimum requirements for the success of the elimination programme (a microfilaraemia prevalence of <1% or an antigenemia prevalence of <2%) [21]. Our case was at a higher risk of infection with LF because of residing in a rural area with a low socioeconomic level and the presence of nearby water collections. In addition, she wasn't used to utilizing any preventive measures against mosquito bites and mentioned a lack of knowledge about this disease. Although infection with LF is usually acquired during childhood, the evident clinical manifestations of the disease may not present until adolescence [27]. The clinical presentation varies widely from asymptomatic infection to marked chronic manifestations. Infected individuals may experience acute episodic attacks of acute filarial lymphangitis (AFL) or acute dermatolymphangioadenitis (ADLA) [28]. AFL episodes are often mild and rarely cause chronic lymphoedema. While ADLA episodes are the most common acute clinical presentation of LF and are more commonly accompanied by considerable pain, fever, and chills [4]. ADLA often occurs after skin trauma or insect bites, with symptoms persisting from three to five days, and is misdiagnosed as cellulitis. ADLA episodes frequently recur numerous times annually, which may later lead to chronic lymphoedema and elephantiasis [3]. In this

patient, several mosquito bites were noticed on the skin of both legs.

She exhibited non-specific symptoms, which may be misdiagnosed by inexperienced medical personnel. The available tools for diagnosis of LF include thick blood film examination, antigen or antibody assays, and PCR techniques that enable the detection of parasite DNA in humans and mosquitoes [(29)]. Recombinant antigen-based filarial antibody assays are highly sensitive markers for LF, even in the absence of antigenemia or prior to microfilarial emergence [(30)]. Besides, the detection of MFs microscopically in Giemsa-stained thick blood smears is considered the standard diagnostic method for LF [(21)].

For blood sampling at any time, the DEC provocative day test can be utilized to detect low quantities of MFs with nocturnal periodicity. If the initial blood film is negative, the blood specimen can be concentrated using Knott's technique, and then the sediment is used to prepare wet smears or stained thick films that are examined microscopically to detect MFs [(3, 31)]. Our case revealed low microfilaremia, so circulating MFs were detected after the use of Knott's concentration technique. Despite being infected, the night blood film examination may be false-negative, especially in case of low parasitic load and during the prepatent period [(4)].

Following the elimination of LF, any newly identified cases should receive a single oral dose of a combination of DEC plus albendazole or ivermectin plus albendazole. DEC is effective against both microfilaria and adult worms, while albendazole has a partial macrofilaricidal effect [(29, 32)]. In our patient, a single oral dose of DEC plus albendazole was efficient in the alleviation of symptoms and clearance of microfilaremia without any recurrence.

In the current study, *C. pipiens* mosquitoes were the only species identified indoors. Likewise, Mostafa et al.[(33)] found that female *C. pipiens* represented the majority of mosquitoes collected from 14 Egyptian governorates, including Sharqia. Moreover, the identified predominance of *C. pipiens* is in accord with El-Naggar et al.[(34)], who reported similar results from Alkorain village, Sharqia governorate.

Moreover, the high abundance of *C. pipiens* indoors is in accord with Gad et al.[(35)] and Zayed et al.[(36)] who reported that C. pipiens had more indoor anthropophilic behaviour compared to other Culex species in Sharqia and Menoufia governorates, Egypt, respectively.

Of all collected female mosquitos, the highest percentage of them were freshly fed (41.2%). Likewise, Dahesh and Ibrahim[(26)] reported that the blood-fed mosquitoes represented the majority of the collected mosquitoes indoors from houses of positive cases in some villages of Giza governorate, Egypt.

In 2000, with reference to WHO recommendations, Egypt was among the pioneers to launch the national LF elimination programme (NLFEP) based on MDA, in which the target population received a single annual dose of DEC (6 mg/kg) in combination with albendazole (400 mg) [(20)]. In 2017, Egypt was the first of the eastern Mediterranean countries to be acknowledged by WHO for attaining the successful elimination of LF [(13)].

Post-MDA surveillance should be conducted periodically or continuously using both human and vector surveillance methods. Human surveillance approaches include nocturnal thick blood film examinations and antigen or antibody detection tests. Vector surveillance could be performed via the detection of filarial parasites in mosquitoes by either mosquito dissection followed by examination for larval stages or the molecular xenomonitoring method [(29)].

Conclusion

The elimination of LF in Alkorain village, Sharqia, Egypt, is still maintained, as the infection prevalence was found to be less

than 1% (WHO-recommended target threshold) in examined humans. Besides, we describe the latest case of bancroftian filariasis in this village after many years of validation of the effective elimination of the disease by the WHO. Based on our case, any case of lower limb swelling of unknown cause should be dealt with a high index of suspicion of LF, especially those in a previously endemic area. Besides, rapid antibody tests for filariasis can serve as a useful screening tool for early detection of filarial infection. In addition, early diagnosis and proper management are always important for reduction of LF-associated morbidity.

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