A Move Towards Defeating Lymphatic Filariasis

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The WHO estimates that over a billion people in more than 80 countries are at risk of contracting lymphatic filariasis (LF) and over 120 million people have already been affected with the disease, with about 40 million people suffering from severe disfigurement and disability.[1] LF, or elephantiasis, is caused by three parasitic filarial worms: *Wuchereria bancrofti*, *Brugia malayi* and *Brugia timori*. Ninety percent of LF infections are attributed to *W. bancrofti* whereas ten percent of LF infections are attributed to *Brugia malayi* and *Brugia timori*.[2] In Thailand, *Wuchereria bancrofti*, is endemic in provinces near the Burma border i.e. Kanjanaburi, Tak and Mahongsorn provinces while *Brugia malayi* is endemic in Narathiwat, Surathani and Nakorn Sri Thammarat provinces in southern Thailand.[3,4] The disease is transmitted through mosquito vectors; e.g. *Culex, Aedes, Anopheles, Mansonia Sp.* When an infected mosquito bites, the infective stage larvae (L3) migrate to the lymphatic system where upon reaching sexual maturity after 6 to 12 months the adult female worms release million of microfilariae into the blood stream.[5] The life cycle is completed when these microfilariae are ingested by mosquito vectors.[6]

Pathology and clinical manifestation

Lymphatic filariasis presents various spectrums of clinical manifestations. The asymptomatic form of infection is most often characterized by the presence in the blood of thousands or millions of microfilariae and adult worms located in the lymphatic system. There are many endemic residents who are presumably inoculated with the infective larvae throughout life, but do not display any outward clinical symptoms. Nevertheless, hidden, internal damage to the kidneys and lymphatic system caused by the parasite are almost always found in all infected individuals.[7]

In LF, the pathology of the lymphatic system is triggered by adult worms in the lymph vessels and lymph nodes. There is little reaction around adult worms until the worms die either naturally or by drug administration, and then inflammation occurs.[8,9] There is an up-regulation of inflammatory cytokines from macrophages in the host when the dying microfilariae and wolbachia-derived molecules, an endosymbiotic bacteria residing in the parasite, are discharged.[10] The clinical symptoms begin with recurrent attacks of filarial fever which typically leads to retrograde lymphangitis (painful, with swelling) and lymphadenitis,[11] lasting for approximately 1 week. While these acute episodes of adenolymphangitis (ADL), are clinically transient in most infected individuals, they can be the starting point for more chronic pathology leading to elephantiasis. Following the lead of the Fifth WHO Expert Committee on Filariasis (1992) and with some minor changes, four stages in the progression of the natural history of chronic lymphedema have been enumerated: viz. 1) reversible edema with no skin folds; 2) pitting edema with some fibrosis; 3) edema together with hardening of the skin (non-pitting) and fibrosis of skin folds; and 4) elephantiasis with irreversible swelling and hard fibrotic tissue.[12] Male patients with lymphoedema are, additionally, at risk for hydrocele (swelling of the scrotal/groin area infected with *W. bancrofti*), who typically have motile adult filariae in supratesticular areas while simultaneously exhibiting few or no microfilariae in the blood concomitant with vigorous specific immune reactions.[13,14] WHO reported in 2004 that among adult residents of endemic areas, 12.5% have clinical manifestations of LE and 21% of men have hydrocele.[15]

Treatment and disability management

The treatment options for filarial nematodes are limited by drug delivery problems and adverse side-effects (produced by the rapid destruction of microfilariae) with no single drug being effective for all clinical disease manifestations. All the antifilarial drugs currently being used (diethylcarbamazine (DEC), ivermectin (IVM), albendazole (ALB)) show a limited macrofilaricidal effect. For instance, after DEC administration, all excised lymphatic nodules showed damaged and degenerating adult worms,[16] while a subsequent report revealed that 41 to 51% of filarial (scrotal) nests of infected men were DEC sensitive; i.e., the filarial dance sign was not detected.[17] These results suggest that DEC is only partially effective against adult worms but readily mediates a suppressive action on microfilariae in the host's immune system.[18,19] A single dose of DEC (6 mg/kg) is as effective as the standard dose (6 mg/kg) given for 12 days.[20] A daily regimen of 1 mg/kg of DEC for one year has been shown to significantly

reduce the number of ADL attacks when contrasted with pre-DEC administration, although an earlier study found insignificant differences in the frequency of attacks between the drug groups (DEC, IVM or placebo) during the treatment and post-treatment phases of the study.[21,22] This latter study further suggested that foot care in conjunction with local antibiotics and anti-fungal agents might be ameliorative in reducing the number of attacks. Moisture between swollen toes promotes fungal infections causing superficial skin lesions, thereby facilitating entry of opportunistic infections, especially while wading through water during the rainy season.[23] By taking steps to prevent bacterial superinfectivity through individual patient management, it is possible to halt and even reverse the inevitable march towards the sequelae of filarial infection, lymphoedema and elephantiasis.[24]

Similar to DEC, a single dose of IVM (400 μg/kg) had no macrofilaricidal efficacy after 9 months of ultrasound examinations, and in fact, 3 live adult worms were surgically removed (8 months post drug administration) from a dilated lymphatic vessel in the scrotal area at the site of prominent filarial dance movements.[25] Even multiple doses at 2 week intervals for 6 months failed to suppress filarial dance movements as monitored by serial ultrasound examinations.[19] Microfilarial density was markedly reduced in all of these men following treatment. Additionally, a single high dose of IVM can suppress microfilaremia for as long as 2 years.[26]

When given in the current regimen of drugs to treat LF, albendazole plays a unique role as it is the only compound which actually destroys adult worms, in addition to clearing microfilaria with an efficacy similar to that of DEC or a combination of ALB/DEC.[27,28] When ALB was co-administered with IVM in a single dose, the results showed high efficacy in clearing mf from night blood and a 77% decrease in antigen levels at the end of 15 months when contrasted with ALB alone or in combination with DEC, although all treatments significantly reduced mf counts.[29] In a subsequent study, ALB + DEC had the greatest activity in clearing mf 24 months post-treatment.[30] Thus, it seems clear that ALB with either IVM or DEC have usefulness in filariasis control programs in areas of high endemicity.

Global program to eliminate lymphatic filariasis (GPELF)

The availability of safe treatment regimens along with rapid diagnostic tools resulted in a global program to eliminate the disease. The two main objectives of the global elimination program are to interrupt transmission of the parasites and to resolve disease manifestations manifested in the suffering and disability of affected patients.[31] Since WHO established as a top priority, in 1997, the reduction and subsequent elimination of lymphatic filariasis (LF) many member countries have taken up the challenge, and have begun successive programs of community-wide mass drug administration (MDA).[32]

The aim of the current GPELF is to achieve worldwide elimination of this vector-borne parasitic disease by the year 2020. To accomplish this, the WHO-sponsored GPELF has recommended that member countries follow yearly mass drug administrations (MDA) in endemic populations for at least 4-6 years.[33] The oral administration of single annual doses of albendazole and diethylcarbamazine (DEC) or ivermectin was aimed at reducting rates of microfilaraemia to below sustainable transmission levels of 1% in areas of high infectivity.[34] Recent work has shown that the decision to stop treatment does not require the complete absence of filarial parasites, but rather the reduction of parasite numbers to such low quantities that transmission will cease.[35] The implication here is that data is necessary for monitoring the nature and magnitude of vector biting and the degree of host infection while simultaneously considering the extent of parasitic elimination. For example, the complexity of the filariasis system dynamics may be seen when new infection rates are lower (than usual). but are due to or occur at greater biting rates in geographical areas of varying parasite eliminations.[36] Thus, if GPELF is to succeed, it is imperative to be able to monitor and measure trends in parasite transmissions and infectivity as a result of anti-parasite interventions.[37,38]

The next phase of the program is to implement the monitoring and evaluation process which is to occur when endemic countries have completed 5-6 rounds of MDA and achieved <1.0% prevalence of microfilaraemia. It is anticipated that these countries will exhibit a gradual decline in the size of the population targeted to receive MDA.[32] In Thailand, all LF endemic areas except Narathiwart province bordering Malasia in the south are moving to this phase.

GPELF continues to make progress. In 2008, nearly 700 million of a total of 1.33 billion who were at risk for lymphatic filariasis were targeted for MDA. Sixty-six of 81 endemic countries have already completed mapping their endemic foci, 13 countries are presently mapping and 2 countries will start mapping. MDA has been implemented in 51 of the 71 endemic countries whereas 20 countries have not yet begun. There are countries where the sociopolitical climate affects the determination whether MDA is to be delivered or not. There are also cautious countries where a widespread *Loa loa* epidemic precludes using DEC (but not IVM) in the MDA for filariasis due to the possibility of severe adverse reactions.[39]

Beside focusing on interrupting transmission, an aforementioned secondary goal of GPELF was the alleviation of the anguish and distress of those already affected. In the context of supportive clinical care, individual counseling and health education both pre- and post treatment are a *sine qua non* for the program success. To assure compliance with drug taking, the targeted population must be afforded the opportunity to learn not only about the transmission and prevention of LF, the dangers of remaining untreated including potential side effects, but also be given information about the benefits of the MDA program.[4]

Diagnostic tools to support GPELF

As with any intervention, close monitoring of progress is necessary to ensure that the MDA program is on track to achieve its goal and to determine when the goal is achieved.[39] Lammie has suggested that GPELF must (a) map geographical areas that require MDA; (b) keep track of the progress in these areas after MDA has been in place; and (c) confirm the absence of infection in these areas.[40] As GPELF programs approach their planned end points, it will be necessary to determine whether the planned interventions were effective in interrupting transmission, and whether MDA can be stopped.[38]

A number of procedures have been used for evaluating a program's effectiveness. An age-tested traditional method for determining the presence of mf has utilized thick blood smears from collected night time blood. The method confers diagnostic specificity, is readily administered with minimal training and is inexpensive. On the other hand, it does not reveal active infections in people with minimal mf counts or those who are amicrofilaraemic.[41] When the rapid ICT card test was developed, it enabled researchers to reliably identify circulating antigens from *Wuchereria bancrofti*. The method was quick (<10 min), minimally inconvenient (100 µl of finger prick blood), easy to use in the field and readily available.[41,42] It has been used extensively as a mapping tool of endemic areas for MDA inclusion.

Many researchers including Thai scientists have tried to develop an assay for the detection of the circulating antigens of *B. malayi*. However, until recently, no such effective antigen detection assay was available for brugian filariasis.[43] An alternative method would be to test IgG4 antibodies that are reactive with recombinant antigens from Brugia species.[44] Various studies have indicated that active filarial infection elevates IgG4 antibodies over appropriate controls, with decrements noted post-treatment.[45,46,47] There are two advantages to using assays for antifilarial antibodies; (a) the time to detect infection is much less than with thick blood smear measurements of microfilaremia or antigenemia, (b) parasitological evaluations are time-point estimates while measuring antibodies returns a cumulative/longitudinal history of the infection.[40] Thus, for all intents and purposes, the antifilarial antibody approach is much more sensitive than the mf thick blood smear approach. Also, further, antifilarial IgG4 assessment could over time provide a useful seroepidemiologic gauge/indicator of the status of lymphatic filaria infection. Both the immunochromatographic rapid dipstick procedure and ELISA versions for detection of antifilarial IgG4 are currently commercially available.[48]

In a recent study, an indirect ELISA for the detection of antifilarial IgG4 was developed by Thai researchers, and a test kit for the diagnosis of lymphatic filariasis has been successively produced and validated for its efficiency.[4,49] This test kit is currently being used in brugian filariasis endemic areas in Narathiwart province, in southern Thailand. As this test kit was developed in Thailand, it is cheaper and, therefore, more accessible than commercial kits produced and sold overseas (Wongkamchai 2009, unpublished data).

Role of monitoring mosquito infection in GPELF

Another tool in evaluating the success of GPELF, is to measure the extent of larval infection in the vector mosquito responsible for the endemicity. The classical method for monitoring mosquito infection is through dissection of each mosquito to detect filarial larvae in the vector population. When the frequency of larval infection in mosquitoes falls to very low levels after many rounds of MDA, large numbers of mosquitoes would be required to reliably estimate the prevalence of such low infection.[50]

The PCR assay is capable of detecting genomic DNA from any stage of the parasite present in the mosquito. The basics of the pool screen assay involve the collection, sorting and pooling of mosquitoes for DNA extraction. The purified parasite DNA is amplified in a PCR amplification procedure using parasite-specific primers; and, finally, the results are analyzed using various statistical algorithms to determine a point estimate of infection prevalence.[51]

Several years after the initial design, several variations of the DNA-extraction method and the PCR detection method were developed.[52,53,54] This led to a multicentre standardization trial in 2002.[55]

More recent modifications included the use of DNA test strips coupled with the pool screen algorithm method for estimating infection rates and the development of real-time PCR for detecting filarial DNA.[56,57] The major advantages to using real-time PCR (as opposed to conventional PCR) was its increased sensitivity with field samples, a decreased possibility of cross-contamination from post-PCR handling and a decreased handling time of post-PCR products, which enables a faster throughput of samples, thereby increasing the efficiency of the assay. An expensive specialized instrument required to detect the PCR product in real time is the one main disadvantage of this technique.

For detecting filarial DNA in a community of mosquitoes, a molecular xenomonitoring procedure uses pool screening DNA methodology. One of the disadvantages is that a large number of mosquitoes must be captured and screened, especially as the parasite prevalence decreases through GPELF efforts. Egypt, France Polynesia, Thailand, Haiti and Papua New Guinea are some of the countries that have successfully used PCR detection of mosquito infections in various field studies, but, the necessary equipment and expertise are not available in all countries and no national PELF programs are currently using this tool for monitoring their activities.[58,59,60,61,62]

Searching for new drugs

The antifilarial drugs currently in use have little or no effect on adult worms. MDA using current antiworm drugs have to be taken for many years to cover the life span of the adult worms, making it difficult to sustain the delivery of the drugs in poor countries. Therefore, new macrofilaricidal drugs are needed.

Recent research has targeted novel drugs with macrofilaricidal and pathology-improving activity. It has been known for more than 30 years that the endosymbiotic bacteria, *Wolbachia* of the order Rickettsiales, are found in the hypodermis of male and female worms, in the oocytes, embryos and larval stages of animal and human filariae.[63,64,65] *Wolbachia* antigens can stimulate the host immune responses that may be associated with the development and progres-sion of pathogenesis of filarial diseases. A low level exposure of the immune system to *Wolbachia* stimuli could occur via the uptake of degenerate larvae released by the females after attacks by the host's phagocytic cells. Upon death of the microfilariae, or adult worms, the immune system would be exposed to a large amount of proinflammatory stimuli, including large numbers of *Wolbachia* which may readily increase the damage to the infected lymph system and cause desensitization in the innate immune system. These events set the stage for an increased susceptibility to opportunistic infections which if left untreated can lead to acute dermatolymphangitis as reflected in lymphoedema and elephantiasis.[66] Thus a continued exposure to acute inflammatory episodes may over time contribute to the pathogenesis of filarial diseases.[67]

The discovery of the essential role of *Wolbachia* in filaria worm fertility and survival heralds a new approach in the use of antibiotics to deplete *Wolbachia* endosymbionts leading to inhibition of worm embryogenesis and eventually viability. Hoerauf administered the antibiotic, doxycycline alone or in combination with IVM to samples of bancroftian filariasis patients.[68] It was found that the antibiotic (200 mg/day for 6 weeks) depleted 96% of the bacteria. After one year there was a 99% reduction in mf which translated to amicrofilaraemia when IVM was added to the antibiotic schedule after 4 months. IVM alone produced a 91% decline in mf. The author's speculated that the mechanism of doxycycline's action resulted in a "predominant blockade of embryogenesis leading to a decline of microfilariae" (p 214). A subsequent study by Debrah indicated that *Wolbachia* depletion was associated with a reduction in the levels of vascular endothelial growth factors (VEGFs) essential for lymphangiogenesis, and both precede a reduction in lymph vessel dilation and improvement of lymphatic disease. Fifty-one (33 microfilaremic and 18 lymphoedema) patients from Ghana received a 6 week regimen of 200 mg/day doxycycline in a double-blind, placebo-controlled trial.[69] Four months after the beginning of treatment, all patients received 150-200 µg/kg of IVM plus 400 mg albendazole. After 2 yrs, all the classic signs of LF were significantly reduced (microfilaremia, antigenemia, the filarial dance sign in the supratesticular lymphatic vessels and the Wolbachia load) in the doxycycline group. At 12 months, the mean levels of the vascular endothelial growth factors (VEGF-C & sVEGFR-3) decreased to endemic normal levels. The improved pathology after 12 months was manifested in better skin texture and a decline in superficial and deep skin folds. The reduction in blood levels of the VEGFs was associated with the amelioration of once dilated supratesticular lymphatic vessels.[69] A recent study specifically targeting hydrocele patients in Ghana found similar results. After doxycycline administration, the mean plasma levels of VEGF-A preceded a reduction of the hydrocele size, concomitant with an improvement in LF pathology.[70]

There has been a spate of confirmatory studies that have utilized an antibiotic's superior activity against parasites that have also targeted the Wolbachia endosymbionts.[71] However, a cautionary note suggests that it is important to determine the threshold, or minimum treatment duration of doxycycline in combination with one of the classically used drugs that retains macrofilaricidal activity and improves lymphatic pathology. A safe and easily administered anti-symbiotic drug combination to kill the bacteria in a shorter period will reduce the time needed for programs to eliminate adult worms from an endemic area.

In conclusion, several strategies have been discussed that are instrumental in seriously limiting the epidemiology of lymphatic filariasis. These include the interruption of transmission using preventive chemotherapy through MDA, the integration of vector management concurrent with MDA, a detailing of effective diagnostic tools and the development of cost-effective test kits, a plea for increased monitoring of outcomes as seen in infectivity trends along with measures of vector biting, the mapping of endemic areas, and new strategies for treatment and morbidity control through antibiotic targeting of the Wolbachia endosymbionts. With an increased emphasis on research through government support and an improving health care delivery system, Thailand is at the forefront of making inroads towards solving many of the problems inherent in the control and eradication of LF.

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