CHAPTER 1

1.0 BACKGROUND

Lymphatic filariasis is a major cause of clinical morbidity and is an impediment to socioeconomic development (Evans *et al*., 1993). More than 120 million people are infected with the filarial nematodes *Wuchereria bancrofti, Brugia malayi*, and *Brugia timori*. The disease is endemic in about 81 tropical countries and is classified as the second most common cause of long-term disability after mental illness (WHO, 2009). *Wuchereria bancrofti* is the most widespread filarial parasite and is estimated to affect approximately 1307 million people in tropical and subtropical areas of Africa, India, Asia, the Pacific islands, and South and Central America (WHO, 2006).

The disease has been considered to be potentially eradicable and as result has been targeted by the World health Organization (WHO) for elimination as a public health problem by the year 2020 (Behbehani, 1998). In the year 2000, national programmes took off in 38 countries where lymphatic filariasis is endemic covering almost 90 million people. The programme is required to meet the target of reaching 350 million people by 2005 and all the 1.2 billion people at risk globally by 2020 (Ottesen, 2000). The main intervention measure recommended for the control of lymphatic filariasis is mass drug treatment of human population to interrupt transmission. The recommended drugs are diethylcabamazine (DEC) and albendazole or in Sub-Sahara Africa, ivermectin and albendazole for a period of four to six years (Ottesen *et al.,* 1997; Ottesen, 2000; Molyneux and Taylor, 2001).

In Sub Saharan Africa, it is estimated that about 512 million people are at risk of the infection and about 28 million are already infected. Of this number, there are 4.6 million

cases of lymphedema and over 10 million cases of hydrocele. These represent about 40% of the global burden of the disease (Michael *et al.,* 1996).

Lymphatic filariasis infections are characterized by a wide range of clinical manifestations. The majority of individual in endemic communities develops microfilaraemia but remain clinically asymptomatic for years. In all endemic areas, a proportion of those individuals may never become symptomatic. When individuals go on to develop clinical disease; it ranges from episodic attacks of adenolymphangitis associated with fever to largely irreversible manifestations such as hydrocele, lymphedema, elephantiasis, and chyluria. In rare instances, infected individuals may develop tropical pulmonary eosinophilia, which is characterized by nocturnal cough and wheezing, low-grade fever, adenopathy, and high-grade eosinophilia (Ottesen, 1980). Although mortality is not associated with the disease, morbidity due to these clinical manifestations is highly significant (Partono, 1987). In addition to time and wages lost from work, the resulting deformities have severe psychosocial impact (Dreyer *et al*., 1997).

1.1 General Overview of Lymphedema

Lymphedema is a physically deforming and socially stigmatizing consequence of filarial infection that affects approximately 16 million people worldwide (Michael *et al.,* 1996). Although the factors responsible for the initiation and progression of filarial lymphedema to its most severe form, elephantiasis, have been debated, recurrent episodes of bacterial acute dermatolymphangioadenitis (ADLA) is believed to play a major role (Olszewski *et al.,* 1997).

Until recently, little could be done to relief the suffering and disability caused by this disease. However, significant advances have been made in understanding both the disease and its control. As a result of these advances, the independent International Task Force for Disease Eradication identified LF in 1993 as one of the six eradicable infectious diseases (Ottesen, 1995). This decision was followed in 1997 by the adoption of Resolution WHA50.29 by the World Health Assembly calling for the worldwide elimination of LF as a public health problem by the year 2020 (WHA, 1997). Following the adoption of this resolution, WHO with support from organizations and donor countries began developing a coalition to eliminate lymphatic filariasis.

In 1998, the coalition was given a powerful boost when Merck and Co., Inc. pledged to expand

its Mectizan Donation Program for Onchocerciasis to cover the treatment of LF in Africa where the two diseases occur together. In the same year, Smith Kline Beecham (SB) announced its commitment to form a unique private-sector or public-sector collaboration with WHO to support the global programme. The aims of the global elimination program was launched with the goal of (i) reducing microfilaraemia levels using filaricidal drugs to a level that is too low to sustain transmission of filarial parasites to humans and (ii) reducing the morbidity associated with chronic filarial disease (Cox, 2000).

Since current anti-filarial drugs do not cure adult worm infections several investigations are being carried out to discover novel anti- filarial drugs with macrofilaricidal effects. One promising area is the use of antibiotics to deplete *Wolbachia* endosymbionts, which leads to the inhibition of worm development, embryogenesis, fertility and viability in

human onchocerciasis (Hoerauf *et al.,* 2000; Hoerauf *et al*., 2001; Debrah *et al*., 2006a) and in LF (Debrah *et al.,* 2007)

In lymphatic filariasis, the establishment of anti-wolbachial therapy could have two advantages: firstly similar to onchocerciasis, adding an anti-wolbachial treatment could also prove cost-effective if the treatment could cause long-term amicrofilaremia in treated persons. Secondly, a major problem with current microfilaricidal mass chemotherapy in lymphatic filariasis is that it does not offer much help to the individuals suffering from the disease (lymphangitis, elephantiasis, and hydrocele) (WHO, 2009) since this group has only low to absent microfilarial blood count. This is due to the fact that pathology is caused by adult worms (unlike microfilariae in onchocerciasis).

Currently, the Mass Drug Administration (MDA) programmes to eliminate lymphatic filariasis mainly offers hygiene and management courses to patients with pathology. While beneficial to various extents, these methods do not reverse the pathology as expected. Frequently, lack of help for those with the pathology results in a reduced compliance in the whole endemic population which is a problem particularly in democracies, because control measures cannot simply be enforced on the population regardless of benefit to individual people. However, high compliance (68%) over decades will be needed in the population if lymphatic filariasis is to be eliminated (Ottesen, 2000).

Various clinical trials have demonstrated that *Wolbachia* can be depleted also from the two major causative agents of human lymphatic filariasis, *Wuchereria bancrofti* and *Brugia malayi*, and that this could eliminate a major factor for the induction of

inflammation (Taylor *et al.*, 2000; Hoerauf *et al*., 2001). If anti-wolbachial treatment proves to be the effective therapeutic approach in the course of time it could bring relief to individuals with signs of disease, with the additional benefit of treating opportunistic bacterial infections associated with episodes of acute lymphangitis (Dreyer *et al.,* 2000). Thus, enhancing compliance and accelerating elimination of lymphatic filariasis.

Successful antibiotic therapy should be the one (i) that would reduce microfilariae slowly (according to their half life), blocking new microfilarial production in adult worms, leading to gradual release of antigen and thus more safety in treatment;(ii) to slow depletion of *Wolbachia* resulting in the prevention of adverse effects due to microfilaricidal drugs and (iii) that will have other effects especially on lymphatic vessels (Pfarr *et al.,* 2009) since improved lymph vessels lead to enhanced lymphatic fluid flow.

Apparently, treatment of pathology in lymphatic filariasis is still empirical and most often unsuccessful because the molecular basis of the pathology is poorly understood. However, recently Debrah *et al*., (2006b; 2009) in pilot studies showed that the disease has genetic propensity, and people who produce more vascular endothelial growth factors (VEGFs) are prone to developing lymphedema. VEGFs are a major mediator of vascular permeability and angiogenesis and play a pivotal role in mediating the development and pathogenesis of many diseases. VEGFs including VEGF-A, VEGF-C, VEGF-D and soluble VEGFs Receptor-3 (sVEGFR-3) are also elicited by endosymbiotic *Wolbachia* in *W. bancrofti*, and targeting the *Wolbachia* with doxycycline leads to amelioration of the legs of lymphedema patients (Debrah *et al.,* 2006b).

Since that pilot study involved a small number of lymphedema patients (15) and most patients do not have active infection but have a lot of exogenous bacteria, it was not clear whether the effects reported was through depletion of *Wolbachia* or other effects of doxycycline on the lymphatic vessels (Pfarr *et al.,* 2009; Fainaru *et al.,* 2008a, 2008b). Thus, there is the need to find out whether the amelioration observed by Debrah *et al*. (2006b) was due to doxycycline acting on endobacteria *Wolbachia* in *W. bancrofti* worms or on exogenous bacteria such as *Streptococcus spp.* found in the folds of lymphedema legs or through any other mechanism. There is also the need to know whether the amelioration observed in the lymphedema legs will be the same in patients with and without active infection. Therefore the aim of the study is to determine the effect of doxycycline which has an anti-wolbachial property and amoxicillin with no anti-wolbachial property on lymphedema patients with and without active infection.

Thus the specific objectives of the study were to:

1. Assess a 6-week course of doxycycline 200mg/day in lymphedema patients
2. Assess a 6-week course of amoxicillin 1000mg/day in lymphedema patients
3. Determine the effects of doxycycline treatment in the stage of lymphedema
4. Compare the effects of doxycycline in both Circulating Filarial Antigen (CFA) positive and negative patients.

CHAPTER 2

2.0 LITERATURE REVIEW

2.1 The parasites and vectors of transmission

Wuchereria bancrofti and *Brugia malayi* are thread -like nematodes, and the adults live within the lumen of lymphatic vessels (Rajan, 1998). Approximately 120 million people in over 81 countries are infected with some form of filariasis (WHO, 2009) with 91% occurring as a result of *W. bancrofti* infection while *B.malayi* sp and *B. timori* infections account for the other 9% (Micheal *et al.,* 1996; Micheal and Bundy, 1997). Of these, approximately 40 million suffer from chronic disease such as lymphedema and hydrocele

In most endemic countries, transmission of the parasite occurs at night by the female *Culex* and *Anopheline* mosquitoes through the process of taking a blood meal from individuals infected with microfilaria (mf) which are millions of larval forms produced by paired adult worms (Burton, 1963).

2.1.1 Life cycle of the parasites

The worms are ovoviviparous, and their larvae are called microfilariae. Lymphatic filariasis (LF) is transmitted by mosquitoes that take up microfilariae in a blood meal. For *W. bancrofti*, man is the exclusive host. Studies have indicated that both *Wuchereria bancrofti* and *Brugia malayi* are about the same size. The female typically measures 4 to 10cm in length and the male 2 to 4 cm. After mating the female worm can release about 10,000 or more offspring per day (www.parasiticdiseases.org). The worms release first-stage larvae, which are known as microfilaria. Each microfilaria measures approximately

270µm by 10µm and contain nuclei that characteristically do not extend to the tip of the tail.

Microfilariae migrate from the lymphatic circulation into the blood stream. However they are typically present in large numbers in the peripheral blood only at night between 22:00hr and 0:00hr in most endemic areas of the world (www.parasiticdiseases.org). Hawking *et al.,* (1962) have suggested nocturnal periodicity to be a result of the microfilaria's penchant for low oxygen tension at which time they are found in the peripheral blood stream or may reflect subtle pH changes in the pulmonary venous circulation during sleep (Hawking, 1962). Picked microfilariae penetrate the stomach wall of the female mosquito and locate to the thoracic flight muscles. There, they undergo three molts, developing into third- stage larvae and become infective after 10 to 20 days of growth and development in the insect muscle tissue (Carme *et al,* 1979).

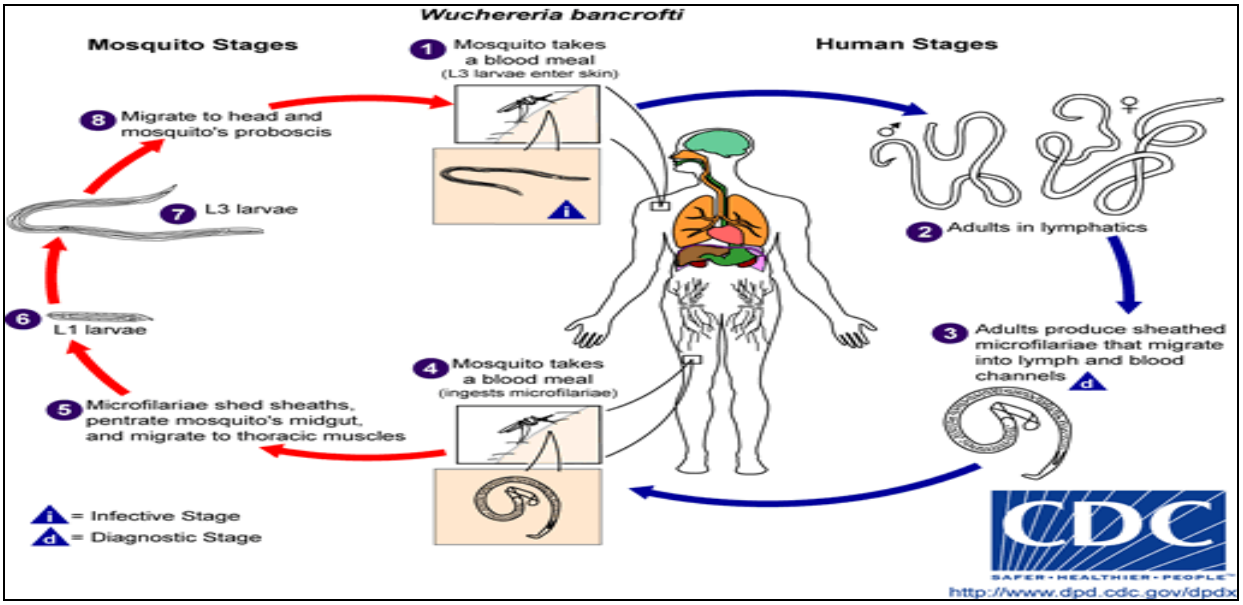


Fig.1.0 Diagram of the life cycle of the parasite; www.cdc.org

Consistent with other investigators, Nanduri and Kazura (1989) have shown that the infective larvae locate the biting mouth parts, and are deposited onto the skin adjacent the bite wound during consumption of a subsequent blood meal. Immature worms migrate through the subcutaneous tissues of the lymphatic vessels. The worms develop into mature adults in about 1 year, and soon after copulation begin shedding microfilariae. The longevity of the adult worm measured by the continuous production of microfilariae is estimated at 5-8years (Carme *et al.*, 1979).

2.2 The spectrum of clinical manifestations

Lymphatic filariasis is a spectral disease, with people living in endemic areas showing a range of clinical manifestations, which vary from one endemic area to another and also differ to some extent on the species of the parasite that is involved (Kumaraswami, 2000)

2.2.1 Filarial attacks

Chronic diseases such as lymphedema and hydrocele are associated with filarial attacks in various ways, depending on the stage of the disease (Dasgupta, 1984; Partono, 1987; Ottesen, 1989; Evans *et al.,* 1993; Ottesen, 1993; Roberts and Janovy, 1997).

2.2.1.1 Acute attacks

In endemic areas acute attacks can occur in amicrofilaraemic or microfilaraemic patients and are common in people with chronic diseases (Evans *et al.,* 1993; Roberts and Janovy, 1997).

There seems to be two distinct causes of these acute attacks. The first, accounting for approximately 10 – 20% of cases, appears to result from the human immune response to

the parasite products released by dead or dying adult worms (Ottesen, 1984; Addiss *et al .,* 1994). The second, and probably clinically more important, is the result of bacterial super-infection of tissues already damaged due to the abnormal lymphatic function caused by filarial infection (Gyapong *et al.,* 1996; Olszewski *et al.,* 1997).

2.2.1.2 Acute filarial lymphangitis (AFL)

Acute manifestations directly caused by live adult worms are usually rare. Acute filarial lymphangitis (AFL) is caused by death of the adult worm (Dreyer *et al.,* 1999). AFL is characterized by lymphangitis that progresses distally or in a 'retrograde' fashion along the lymphatic vessel, producing a palpable 'cord'. Rarely, AFL is accompanied by mild fever, headache, and malaise. Distal lymphedema may occur, but is usually mild and reversible, that is, self-limited according to (Dreyer *et al.,* 1999).

2.2.1.3 Acute dermatolymphangioadenitis (ADLA)

Aetiology of ADLA in filariasis-endemic areas comes from the distinctive clinical signs and symptoms, isolation of bacteria at the time of the acute episode, and changes in antibody titres between acute and convalescent serum specimens (Suma *et al*., 1997; Pani *et al.,* 1995). The bacteria believed to be most frequently associated with ADLA are Group A *Streptococcus*. Other bacteria are often found in cultures, including those that are usually regarded as non-pathogenic (Olszewski and Jamal*,* 1994; Olszewski *et al*., 1997; 1999). Existing evidence indicates that the immune system may amplify or modulate ADLA. Little is known about the antimicrobial sensitivity of bacteria isolated from persons with ADLA in filariasis-endemic areas. Available experience suggests that

the organisms most commonly involved are sensitive to penicillin; thus, penicillin is usually recommended for treatment (WHO, 2006; Bonnetblanc and Bedane, 2003).

2.2.1.4 Lymphedema and Elephantiasis

Lymphedema is characterized by a chronic disfiguring and disabling swelling of one or several part(s) of the body such as limbs, genitalia owing to insufficient lymphatic drainage (Rockson, 2001). According to Karkkainen *et al.,* (2002) accumulation of stagnant, protein- rich fluid into the interstitial matrix between cells reduces the delivery of oxygen and other molecules to cells and attenuates immune responses in lymphedematous tissues.

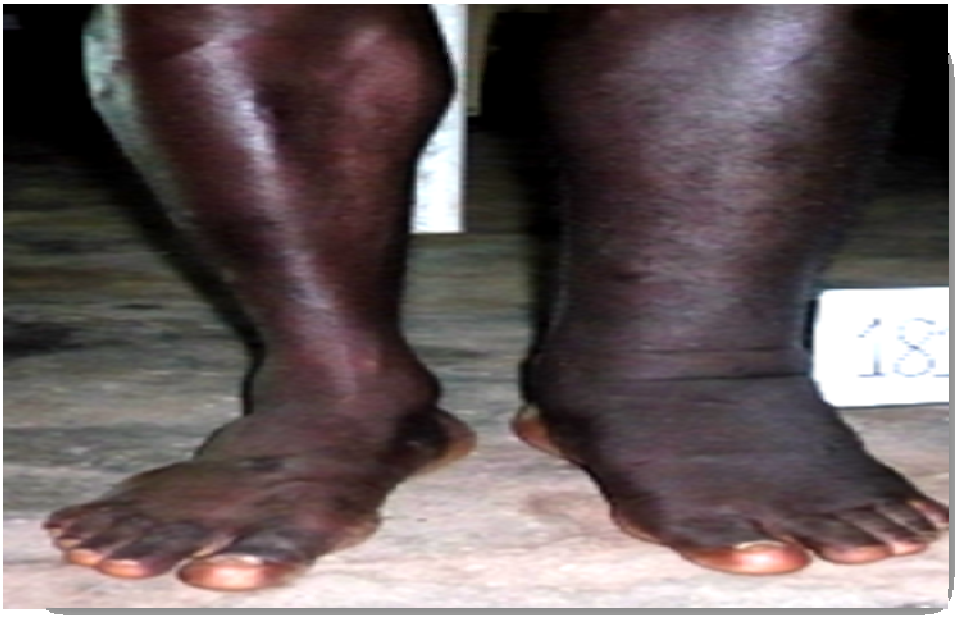


Plate 1.0 The onset of folds development in lymphedema

In the initial stages the swelling can be best obscured around the ankle which gradually spreads to the back of the foot. The leg can become three times the original size. It is believed that parasites (adult worms) living in the lymphatic channels cause damage to

the vessels, perhaps by producing mediators that cause vessel dilatation or inhibited contractility (Olszewski *et al .,* 1993). In addition to dilatation and contractility damage to the lymphatics, occlusion of the lumen by dead worms and host inflammatory immune responses can also be associated with lymphedema and elephantiasis (Ottesen, 1980). Chronic lymphatic pathology is not merely the result of mechanic flow obstruction but there is evidence of the role of inflammatory responses in its pathogenesis (Olszewski *et al.*, 1993). In contrast, hydrocoele, one of the most frequent manifestations of bancroftian filariasis, often occurs without any concomitant inflammatory reaction (Dreyer and Piessens, 2002).

2.3 Mechanisms of pathogenesis in lymphedema

Pathogenesis of lymphedema is far from being clear. Direct damage to the lymphatic vessels from the worms, inflammatory and immune-mediated damage, induced lymphatic endothelial cell (LEC) proliferation and differentiation (Bennuru and Nutman, 2009), superinfections and lymphatic flow obstruction have all been proposed (Tabibiazar *et al.,* 2006). Probably all concur, concomitantly or in different points in time, to the development of the pathology. Moreover, it must not be excluded that the same pathologic outcome could be reached by different mechanisms (Dreyer *et al*., 2000; Dreyer and Piessens, 2002).

Although, it is difficult to establish a straight forward pathway for the onset and progress of lymphedema, the presence of the adult worms located in the vessels and lymph nodes are known to be essential triggers for the process of pathogenesis. From immunohistology it is believed that no or few reactions are seen around the live adult worms. Dreyer *et al* (1996) suggested sub-clinical lymphangiectasia to be the most

common reaction that signifies the presence of living worms which are easily observed in childhood in humans.

In contrary, several studies have indicated the presence of heightened inflammatory processes after the death of the adult worm and broad variety of circumstances such as factors depending on the parasite and the host, natural attrition or by inducement from drugs may lead to worm death (Figueredo-Silva *et al*., 1996; Figueredo-Silva *et al*., 2002). Different from the live worms which are found completely free in the lumen of the lymphatic vessels, Figueredo-Silva *et al*., (2002) reported damaged worms sometimes appear connected to vascular wall by strands of fibrin-like material.

Antigenic stimulation is believed to be one of the probably early events following lymph vessel dilation, which takes place in the presence of live adult worms, when offspring larvae are being released. Studies show that the exposure of the phagocytes to filarial antigens is accompanied by triggering of the innate immune system (Brattig *et al.,* 2000; 2004); with the release of proinflammatory cytokines but also the release of molecules that promotes lymphangiogenesis. In addition to antigens from the worm itself, previous data implicate *Wolbachia* endosymbionts as major player in LE pathogenesis (Taylor *et al.,* 2000) as their depletion by doxycycline leads to drastic reduction of proinflammatory cytokines and lymphangiogenesis molecules resulting in reduction in lymph vessel dilation (Debrah *et al.,* 2006b)

However, studies by Bennuru and Nutman (2009) recently have suggested that filarial parasites directly induce lymphangiogenesis and lymphatic differentiation and provide insight into the mechanism underlying the pathology seen in lymphatic filariasis.

2.3.1 Economic and social burden due to lymphedema

The social and economic impacts of lymphedema have now been well documented (WHO, 2009). Lymphedema is known to be a cause of physical disfigurement, social stigma, loss of self-esteem, lowered employment opportunity, and family discord. The pathology is known to cause massive economic loss due to the direct costs of disease treatment, loss of work due to episodic attacks of ADL, lowered productivity, labour input, and lowered input into economic and household activities (WHO, 2002).

In addition to the economic impact caused by the disease, data from Ghana show that lymphedema imposes a heavy psychosocial burden upon affected individuals (Evans *et al.,* 1993, Gyapong *et al.,* 1998). A cross-sectional study in Togo suggests high risk of depression is consistent in patients with higher stages of lymphedema than among subjects with earlier stages (Stephanie *et al.,* 2007). In coastal Ghana such people are subjected to teasing and considered to be unsuitable marriage partners. Those who do marry have a higher than normal divorce rate (Ahorlu *et al.,* 1999).

2.4 CHEMOTHERAPY

2.4.1 Ivermectin

Ivermectin is an 80:20 combination of avermectin B1a and avermectin B1b, macrocyclic lactones produced by the actinomycete, *Streptomyces avermitilis* (Goa *et al.,* 1991).

The drug has been used extensively for the control of a wide variety of parasites in farm and domestic animals (Horton, 2000).

In humans, ivermectin was first studied in Senegal by Aziz and colleagues (1982). Ivermectin has been the drug of choice for the treatment of Onchocerciasis and LF

(Ottesen, 1984). However, microfilariae quickly reappear few months after a single dose (Dunyo *et al.,* 2000; Plasier *et al*., 2000). Since ivermectin is only a microfilaricidal agent, treatment is suppressive rather than curative. Therefore, it may need to be taken for at least the lifespan of adult worms (up to 15 years) (Whitworth *et al.,* 1991).

2.4.2 Mechanism of action, pharmacokinetics, safety and efficacy of ivermectin

The mechanism of action of ivermectin probably involves opening of the glutamate- and GABA-gated chloride ion channels of nematode neurons, thereby impairing their normal function and affecting feeding, motility and uterine activity (Campbell, 1991). The interruption of nerve transmission causes paralysis followed by death. In mammals, these neurotransmitters and receptors are confined to the central nervous system. In normal conditions the drug does not cross the blood-brain barrier and does not penetrate the central nervous system (Burkhart and Burkhart , 1999).

Side reactions to the drug are limited almost exclusively to the consequences of inflammatory reactions to dead parasites, particularly the microfilariae. Indeed, it has been shown quantitatively that both the incidence and intensity of these reactions are directly proportional to individuals' pretreatment microfilarial loads (Francis *et al.,* 1985).

2.4.3 Diethylcarbamazine

Diethylcarbamazine (DEC) was developed in 1947 as a derivative of the antiparasitic drug, piperazine (Ottesen, 1985). This partial macrofilaricidal drug was the therapeutic agent for the treatment of most forms of filariasis such as LF, loasis, and onchocerciasis (Ottesen, 1987). Most likely related to it being a derivative of piperazine,

diethylcarbamazine is effective against a number of human helminth parasites, including most filarial parasites.

2.4.4 Mechanism of action, pharmacokinetics, safety and efficacy of DEC

The mechanisms of action of diethylcarbamazine against the filariae, thus far identified focuses on two main areas: the effects of the drug itself upon the parasite and the facilitation of host- parasite interactions. DEC has been shown to over-stimulate parasite neuromuscular systems and increase motility, thus inhibit vital parasite metabolic systems and activate complement on the parasite's surface membrane (Maizels and Denham, 1992). DEC has been observed to induce severe and sometimes life threatening adverse reactions on individuals living in areas where there is co-endemicity of onchocerciasis and /or loasis with *W. bancrofti* as in Africa (Ottesen and Ramachandran, 1995). There is also dermal and systemic effects especially in patients with onchocerciasis in which the response has been termed the mazzoti reaction (Francis *et al.,* 1985). The adverse reactions to filarial treatment are known to be severe and even fatal in heavily infected individuals and impede the community participation in control programmes (Taylor *et al.,* 2000).

2.4.5 Albendazole

Albendazole is a benzimidazole carbamate which inhibits the polymerization of tubulin (Lacey *et al.,* 1987; Oxberry *et al*., 2001). It has been effectively used for the treatment of intestinal helminths for almost 20 years but it has only recently been used as an anti-filarial drug (Horton *et al*, 2002). It exhibits larvicidal, ovicidal and vermicidal activity and is thought to exert its antihelminthic effects by inhibiting tubulin polymerization and

disrupting the worm's metabolism. Studies by Awadzi *et al* (1995) indicated that the drug mainly appears to have little embryocidal and/or adulticidal effects which have also been reported in some parts of India (Pani *et al.,* 2002) but not in Africa. Ivermectin in combination with albendazole showed no evidence of reducing microfilaria intensities or in clearing infection when compared to ivermectin alone in Africa (Ismail *et al.,* 1998, Dunyo *et al.,* 2000)

2.4.6 Mechanism of action, pharmacokinetics, safety and efficacy of albendazole

Albendazole binds to beta-tubulin thereby preventing microtubule assembly. Other potential mechanisms are that albendazole inhibits fumarate reductase (parasite specific) and causes a decreasing level of NADH as well as degradation of endoplasmic reticulum and mitochondria thereby decreasing production of ATP (Lacey *et al.,* 1987).

First introduced for human use in 1982, single-dose albendazole (usually 400 mg) has been administered to at least 800 million people for the treatment or control of helminth infections (Horton, 2002). Despite this enormous exposure of individuals and populations, there have been few, if any, serious adverse events that can be definitely related to the drug itself.

2.4.7 Lymphedema and antibiotics- tetracycline in focus

Tetracyclines are concentrated by susceptible bacteria and inhibit the bacterial protein synthesis. The site of action is the bacterial ribosome (Goe *et al.,* 2004). The antibiotic gain access to the ribosome directly by passing through the lipid bi-layer and using active transport system pumps the drug through the cytoplasmic membrane. Once it gets access to the bacterial cells, it binds principally to the 30S subunits and prevents access of

aminoacyl t- RNA to the acceptor site A on the mRNA ribosome complex. This prevents the addition of amino acid to the growing chain (Goe *et al.,* 2004)

2.4.8 Absorption and extraction of tetracycline

Most of the tetracyclines are adequately but completely absorbed from the gastrointestinal tracts (Goe *et al.,* 2004). Absorption of tetracycline is impaired by the concurrent ingestion of dairy product. All tetracyclines are concentrated in the liver and excreted by way of the bile into intestines from which they are partially reabsorbed. All the tetracyclines are excreted in the urine and feaces, the primary route being the kidney (Goe *et al.,* 2004).

2.4.9 Contraindications of tetracyclines

The tetracyclines produce varying degrees of gastrointestinal upset (nausea, vomiting and diarrhea) (Goe *et al.,* 2004), skin rashes, mucous membrane lesions, and fever in many patients particularly when administration is prolonged and dosage high. They are contraindicated in people with hepatic and renal impairment, breastfeeding mothers, and people with systemic lupus erythematosus (SLE) (Goe *et al.,* 2004). They are deposited in bony structures and teeth, particularly in the fetus and during the first 6 years of life. Discoloration and fluorescence of the teeth occurs in newborn if tetracyclines are taken for prolonged period by pregnant women. (Goe *et al.,* 2004)

2.4.10 Anti-wolbachial treatment in lymphatic filariasis-lymphedema

The search for new drugs having long term sterilizing and macrofilaricidal activity on the worms has been ongoing for some time now. Despite several attempts none of the

development made was field applicable (Hoerauf *et al*., 2002). Unconventional approaches to orthodox chemotherapy emerged as endosymbionts filariae *Wolbachia* were detected as prospective drug target (Townson *et al.,* 2000; Hoerauf *et al*., 2001; 2003). Over the past decade targeting endobacteria *Wolbachia* with doxycycline satisfy the long term sterilizing principle.

The availability of safe drug doxycycline has encouraged clinical investigators to test their hypothesis that elimination of *Wolbachia* is one of the potential approaches in reducing the human filarial infections. The first clinical trials were done in people having onchocerciasis infections. A 6- week course of daily doxycycline treatment (100 mg/day) depleted *Wolbachia* in worms, and caused extensive degeneration of embryos by 4 months post-treatment (Hoerauf *et al.,* 2000). Data from the study shows that worms became sterile after the loss of *Wolbachia*, and infected individuals also had significantly fewer or no microfilaridermia (Hoerauf *et al.,* 2000).

Hoerauf *et al* (2001) again demonstrated in human onchocerciasis that antibiotic therapy was effective against *Wolbachia* and other possible effects on lymphatic vessels and therefore could be superior in efficacy to conventional microfilaricidal drugs such as ivermectin, the only drug currently available for mass treatment. This is because anti-wolbachial therapy led to a sustained sterilization of adult worms and lack of microfilaridermia in patients over a long period (Hoerauf *et al*., 2001), in contrast to ivermectin where microfilariae reappear within a few months in quantities sufficient for transmission to continue as embryogenesis in the long-lived (11-15 years) adult worms is undisturbed (Awadzi *et al.,* 1995). Similar effects were observed in *W. bancrofti*-infected patients after multiple doses of doxycycline (200 mg/day for 6wk) (Hoerauf *et*

al., 2003, Debrah *et al.,* 2006b). In this study, patients were treated with doxycycline followed by a single dose of ivermectin. Doxycycline treatment alone reduced *Wolbachia* numbers (96%) after 4 months of treatment, followed by 99 % reductions in number of microfilariae by one year of treatment. These results and others demonstrated by various researchers around the globe prove that doxycycline is a suitable drug in attempt to eliminating lymphatic filariasis (Debrah *et al.,* 2006b; 2007b; 2009).

2.4.11 The role of *Wolbachia* in the development of lymphedema

The role of *Wolbachia* in lymphedema is less clear and still under investigation. Both presence of proinflammatory cytokines in liquid from lymphedema and positive correlation of the frequency of acute attacks with development of lymphedema and elephantiais (Dreyer and Piessens, 2002; Pani *et al*., 1995) indicate that inflammation is an ongoing process in limb chronic pathology. *Wolbachia* could contribute to this process in various ways. As described before, *Wolbachia* can directly induce an inflammatory response from cells of the innate immune system; moreover the finding of Punkosdy *et al*. (2003) that levels of anti-WSP antibodies were negatively correlated with duration of lymphoedema suggests a direct role for *Wolbachia*.

For a decade, several experimental works regarding contribution of *Wolbachia* to filarial disease came from studies on the molecular pathogenesis of filarial-induced inflammation which showed that bacterial lipopolysaccharide (LPS)-like molecules derived from *Wolbachia* are responsible for the activation of innate inflammatory responses (Taylor *et al.,* 2000). In human onchocerciasis, *Wolbachia* have being implicated as the essential triggers of the neutrophil response around worms (Brattig *et al.,* 2001). The release of bacteria or LPS-like molecules following death of parasites after ivermectin treatment

suggested that these mechanisms may be responsible for the adverse inflammatory reaction to filarial chemotherapy (Taylor *et al.,* 2000), as often experienced in individuals with a high parasite load. Another suggested mechanism, not mutually exclusive with that described so far, has been proposed by Punkosdy *et al.* (2003). They suggest that *Wolbachia* may trigger a shift in host response to a Th1 response to both filarial and non-filarial antigens, therefore heightened the reactivity to bacterial antigens. Nonetheless, since *Wolbachia* has been implicated as one of the major causes of filarial pathology, antibiotic therapy in addition to anti-nematode activity, may as well be useful in preventing filarial pathology, as well as reducing adverse side effects after microfilaricidal drugs.

2.5 Vascular endothelial growth factor (VEGFs) and lymphedema development

VEGF, discovered in 1989, is a major mediator of both vasculogenesis and angiogenesis (Ferrara, 2000). Studies on the molecular mechanisms controlling the lymphatic vessels have shown that vascular endothelial growth factors C (VEGF-C) and VEGF-D specifically control lymphangiogenesis in humans (Korpelainen *et al.,* 1998; Achen *et al.,* 1998) by activating the VEGF receptor-3 (VEGFR-3) (Veikkola *et al.,* 2001; Jeltsch *et al.,* 1997), which is principally restricted to the lymphatic endothelium in adults (Kaipainen *et al.,* 1995; Kukk *et al.,* 1996). In animal models, overexpression of VEGF-C in the skin of transgenic mice resulted in lymphatic endothelial proliferation and dilation

of lymph vessels (Jeltsch *et al.,* 1997) with a resemblance to lymphatics infected with filarial parasites (Taylor *et al.,* 2001). Additional evidence for the role of VEGF-C/VEGFR-3 in the pathogenesis of lymphatic dilation and lymphedema stems from experimental studies in transgenic (Makinen *et al.,* 2001) as well as in humans by Debrah *et al.,* (2006b; 2007a; 2009)

CHAPTER 3

3.0 Materials and Methods

3.1 Study Area

The study was conducted in 25 communities in the Nzema East and the Ahanta West Districts in the Western Region of Ghana which are highly endemic for lymphatic filariasis (Dunyo *et al*., 1996; Hoerauf *et al.,* 2003). Dunyo *et al* (1996) identified these communities with microfilariae prevalence ranging from 5 to 20% and an average population of 500-800 (Ahorlu *et al.,* 2001). The main occupations of the settlers are fishing, farming, charcoal burning and other small scale businesses.

Houses found in these study communities were made of mud-for walls, with dried coconut leaves and bamboo sticks for roofing. Most inhabitants in the communities close to the sea sleep at the seashore without bed nets, having thus a higher rate of exposure to mosquito bites particularly during high temperature seasons.

Plate 2.0 Map of the study villages. Source: ENCARTA, 2010

Most of the communities are located along various rivers, lagoons and mangroves which serve as breeding sites for anopheles mosquito, the main vector of the transmission of the

disease. Almost all the studied communities lack health centres, therefore, medical treatment were usually sought from nearby towns like Nkroful, Dixcove, Axim, Ayinase Agyambra, Asemasa and Essiama all in the Western Region of Ghana. The study villages included Ampatano, Asemasa, Asemkow, Butre, Ehuntumano, Busua, Dixcove, Achowa, New Akwidaa all of the Ahanta West District while Cape Three Points, Agyambra, Miamia, Aiyinase, Asasetre, A.B Bokazo, Teleku Bokazu, Axim, Awiebo, Bakanta, Apataim, Ampain, Azulenuano, Ndatiem, Salma, Sanwoma are in the Nzema East District.

3.2 Ethical clearance

Ethical clearance was given by Committee on Human Research, Publication and Ethics (CHRPE) of Komfo Anokye Teaching Hospital (KATH) and Kwame Nkrumah University of Science and Technology (KNUST). Permission to conduct the studies in the selected communities was sought from the Ahanta West and Nzema East Districts Health Administrations.

At the beginning of the study, meetings were held in all the communities with opinion leaders to explain the purposes and procedures of the study in the local languages, ie Nzema, Ahanta, and Twi. Informed consents were obtained from each participant.

3.3 Selection of villages

Participants of the study comprised mainly Ahanta and Nzema speaking people from Ahanta West and Nzema East districts respectively. The criteria for selecting these communities for the study were based on the assessment of the prevalence of the disease in these areas by Dunyo *et al* (1996). Secondly, most of the people were local farmers

and fishermen and had no plans of relocating within the time frame of the study (2 years). Lastly, community sizes were relatively small facilitating easy daily drug administration as well as those known to have good compliance to other studies alike confirmed by the village health workers.

3.4 Selection of lymphedema Patients

3.4.1 Exclusion and inclusion criteria

Individuals included in the study exhibited the clinical state of the disease (lymphedema) and were taken through careful examination of the feet. Questionnaires (Appendix I and II) were administered to find out for instance, experience of attacks, swelling and reversal of the affected leg, peelings, swelling of lymph nodes, frequent fever etc. The patients who qualified were registered. Basic personal data such as name, sex, age, occupation, ivermectin treatment status were recorded. The age limit for inclusion ranged between 18-60 years.

Individuals excluded from the study included those with mental disorders, pregnant women, breastfeeding mothers and those with other acute and chronic diseases such as convulsion, hypertensive, diabetics and those who had abnormal hepatic and renal status (GPT >40µ/l, GGT > 45µ/l, and creatinine >126µmol/l) measured by dipstick chemistry (REFLOTRON). Pregnancy test was done for all the women and those who were positive were excluded from the study.

3.5 Clinical chemistry test

In assessing the patients' kidney and liver functions, clinical biochemistry tests were done using stick- technology by the Reflotron® system (Boehringer Mannheim,

Germany, Roche). Blood samples were collected and centrifuged to separate plasma from blood cells. About 200µl of each patient's plasma was pipetted into 1.8ml eppendorf tube bearing the individual number (ID no) of each patient. Subsequently, 30µl plasma was pipetted from the eppendorf tube unto the various Reflotron® test strips using a Reflotron® pipette according to the protocol of the manufacturer. The tests were carried out at the KCCR field laboratory at Essiama Health Centre. Parameters measured by the stick technology were glutamate-pyruvate –transaminase (GPT), gamma- glutamyl-transpeptidase (GGT) and creatinine (CREA).

3.6 Determination of circulating filarial antigens

In determining adult worm vitality, the presence of circulating filarial antigen (CFA) in the blood was assessed quantitatively using the Og4C3 ELISA (More and Copeman, 1990), according to the specifications given by the manufacturer (TropBio, Townsville, Australia). Following the test protocol, 100µl plasma was diluted in 300µl sample diluent. The samples were then boiled to liberate the heat-stable antigen that is detected in the test. After centrifugation, 50µl supernatant was added to each well of microtiter plates coated with the Og4C3 monoclonal antibody. The test was developed as a standard ELISA using rabbit anti-*Onchocerca* antiserum, followed by an overnight incubation with peroxidase-conjugated anti-rabbit antiserum and chromogen. The test is set up not for a fully quantitative but for a semi-quantitative analysis, whereby the color intensity for a given serum or plasma sample (diluted 1:4) is compared to standard values pre-diluted by the manufacturer (levels: 32,000, 8,192, 2,048, 512, 128, 32, <10 antigen units, corresponding to titer groups 7, 6, 5, 4, 3, 2 1, respectively). Titer group 3 is considered

equivocal, groups 2 and 1 are considered antigen negative. Colour intensity of the test was measured at wavelength of 450nm.

3.7 Assessment of microfilaraemia in lymphedema

Patients recruited for the study were asked to donate 10ml of venous blood which was taking between 22.00 and 24.00 hours. Subsequently, 100µl of each patient's blood was diluted in a 900µl of 3% acetic acid. This was then counted in a sedgewick counting chamber for microfilarial load in each patient and expressed as Mf/ml of blood (Hoerauf *et al.,* 2003). As confirmation 100µl or 1000µl (if the sample had very low Sedgewick count) of blood was diluted in aqua desk and filtered through 5µm Millipore filters (Whatman's nucleopore, Kent, UK). The filters were stained with Giemsa and Mf load counted.

3.8 Limb measurement of lymphedema patients

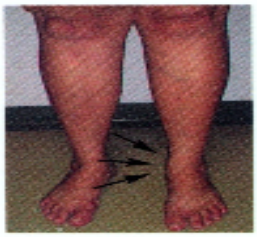
The circumference of both affected and normal legs were measured using a measuring tape at 5 different points. Measurements were taken at 10cm from the large toe, and 12cm, 20cm, 30cm, 50cm and 60cm from the sole of the foot as described by (Kumaraswami, 2000) and the average of the five measurements calculated and taken as the mean circumference of the leg. Measurements were done at pretreatment and subsequent follow- up time points.

Plate 3.0 Measuring a lymphedema patient's leg.

3.9 Staging lymphedema legs

Staging of lymphedema was performed along the guidelines of ''Basic Lymphedema Management'' (Dreyer *et al.,* 2002b). Stage1: Swelling that is reversible overnight, Stage 2: Swelling that is not reversible overnight, Stage 3: Appearance of shallow skin folds at the ankle of the foot, Stage 4: Presence of knobs (lumps or protrusions), Stage 5: Presence of deep skin folds plus knobs, Stage 6: Deep skin folds plus knobs and mossy lesions, and Stage 7: Parameters mentioned above plus patient inability to perform routine daily activities (Plate 4)

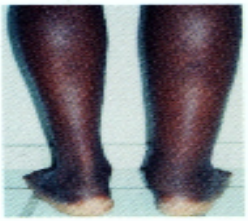
LYMPHOEDEMA STAGING AND MANAGEMENT FOR FILARIASIS–ENDEMIC AREAS



Stage 1

Characteristic feature: Swelling is reversible (goes away) overnight.

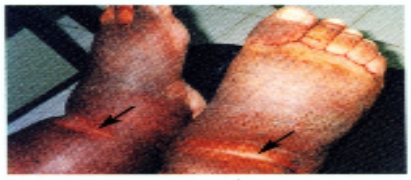
In stage 1 lymphoedema, the swelling increases during the day and goes away overnight when the patient lies flat in bed. To accurately classify lymphoedema in patients with stage 1 disease, it is best to examine the leg in the late afternoon, when the swelling is most visible, and again in early morning, to see that the swelling is gone. If there is swelling in both legs, it may be necessary to rely on the patient's report of normal-size legs in the morning, because comparison with the patient's "normal" leg is not possible.



Stage 2

Characteristic feature: Swelling is not reversible (doesn't go away) overnight.

The main difference between stage 2 lymphoedema and stage 1 is that the swelling does not go away without lymphoedema management. Occasionally, patients with stage 2 lymphoedema will have acute attacks. They also may have entry lesions between the toes, and a mild bad odor.



Stage 3

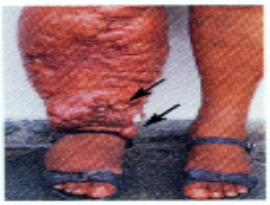
Characteristic feature: Shallow skin folds.

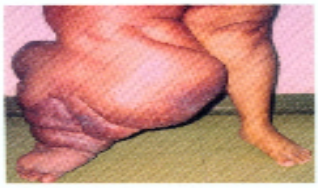
The principal feature of stage 3 lymphoedema is the presence of one or more shallow skin folds. Shallow folds are those in which the base of the fold can be seen when the patient moves the leg or foot so that the fold "opens up". Even very thin lines or creases, which are not seen on normal legs, are considered shallow folds. Early shallow folds are much easier to see when the patient is standing. Thus, it is important to have the patient standing when you are staging the lymphoedema.

Stage 4

Characteristic feature: Knobs.

The main feature of stage 4 lymphoedema is the presence of knobs. Knobs are bumps, lumps, or protrusions of the skin. The importance of knobs comes from the fact that they predispose the leg to further trauma and, therefore, to additional entry lesions, especially if the skin at the site of the knob is less sensitive than the surrounding skin.





Stage 5

Characteristic feature: Deep skin folds.

The presence of one or more deep skin folds is the main feature of stage 5 lymphoedema. Deep folds are those whose base cannot be seen when the patient moves the leg or foot so that the fold "opens up"; rather, the base of the fold can be seen only when the edges are actively separated by hand.

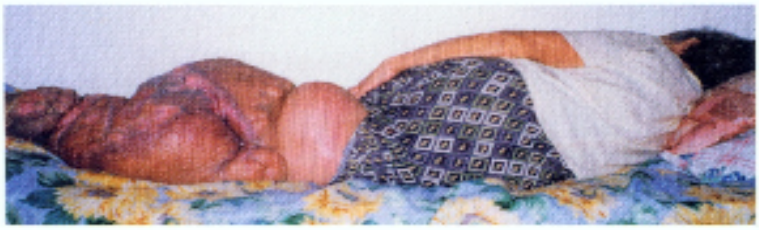
Stage 6

Characteristic feature: Mossy lesions.

On the surface of the skin (especially the upper surface of the toes), very small elongated or rounded small growths may develop. They are usually clustered together, giving rise to the peculiar appearance of "mossy lesions". When located on the foot, this condition is known as "mossy foot". Rarely, these lesions can appear on the leg.

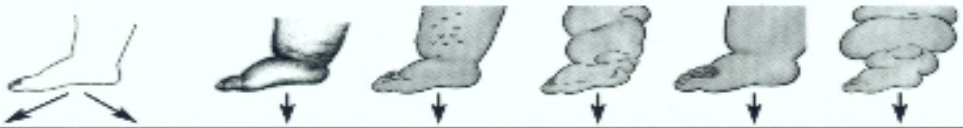
Stage 7

Characteristic feature: Unable to care for self or perform daily activities.



The patient is unable to adequately or independently perform routine daily activities such as walking, bathing, or cooking, etc. Patients with stage 7 lymphoedema have frequent acute attacks and large legs, usually with deep folds. They always have entry lesions between the toes and skin folds. The bad odor is very strong. Wounds in the skin are commonly present, and lymphoedema extends above the knee in most patients. The principal feature of stage 7 lymphoedema is that the patient cannot perform daily activities. Assistance from the family and the health care system is needed.

LYMPHOEDEMA MANAGEMENT, BY STAGE



Treatment Component	Stage 1	Stage 2	Stage 3	Stage 4	Stage 5	Stage 6	Stage 7
Hygiene (washing and drying)	Yes (ideally at night)	Yes (ideally at night)	Yes (ideally at night)	Yes (ideally at night)	Yes (twice a day if possible)	Yes (twice a day if possible)	Yes (twice a day if possible)
Care of entry lesions	If present	If present	If present	If present	If present	If present	If present
Exercise	Yes	Yes	Yes	Yes	If possible	If possible	If possible
Elevation	Usually not necessary	At night	Day and night	Day and night	Day and night	Day and night if possible	Day and night if possible
Prophylactic creams	No	No	Usually not necessary	Usually not necessary	Usually necessary	Necessary	Necessary
Prophylatic systemic antibiotics (send to doctor)	No	No	No	Usually not necessary	Usually necessary (if acute attacks persist)	Necessary	Necessary
Cosmetic surgery	Not applicable	Not applicable	Not applicable	If medically indicated	If medically indicated	If medically indicated	If medically indicated

Plate 4.0 Stages of lymphedema legs (Dreyer *et al.*, 2002b)

3.10 Ultrasound examination

Ultrasound examinations was done by a portable hand carried machine (SonoSite 180 Plus®) equipped with an L 38 mm, 5-10 MHz linear transducer plus Pulse Wave- and Colour Doppler device. It is a non invasive method used mainly for the detection of filarial dance sign (Amaral *et al.,* 1994; Mand *et al,* 2003). This technique was used to measure the skin thickness at the *Malleolus lateralis* and *Malleolus medialis* at all time points.

3.11 Treatment regimen

Patients were randomized into three treatment arms, thus doxycycline, amoxicillin and placebo. Subjects in the doxycycline arm received 2x100-mg capsules of doxycycline (Vibromycin; Pfizer). Participants in the amoxicillin group received 2x 500-mg of amoxicillin and the rest received matching placebo supplied by the manufacturer daily for a total of 42 days (6 weeks) in a daily observed treatment (DOT). Six months after the start of treatment, all participants received an oral 400-mg dose of albendazole and a 150-µg/kg dose of ivermectin (Mectizan; Merck). Randomization was performed using computer-generated random number software (StatView).

3.12 Follow -up examinations of patients

Patients were reexamined for microfilarial load at 6, 12, and 24 months after treatment. CFA as well as USG, circumference leg measurement and staging were assessed during these time points. Adverse effects were assessed during the drug treatment period.

3.13 Statistical analysis

Statistical evaluations were done using descriptive statistics for frequency distribution, Chi-square analysis, non-parametric tests for paired (Wilcoxon test), unpaired (Mann-Whitney-U test) and Friedman test for paired samples from more than 2 observation time points. Leg and USG measurements were summarized as geometric mean and analysed using the Wilcoxon signed rank test and changes in (CFA) antigenaemia levels were computed as percentages from baseline and later analyzed between the groups at subsequent follow up time points by Mann-Whitney-U, and Kruskal-Wallis. A two-tailed p-value lower than $p=0.05$ was considered statistically significant. For all analysis statistics program Statview® and SPSS were employed.

CHAPTER 4

4.0 RESULTS

4.1 Adherence to treatment, stratification and drop-out

A total of 180 lymphedema patients were present for recruitment the study. Of these, 31 representing (17%) were excluded for not meeting the inclusion criteria. A total of 149 lymphedema patients were stratified and treated according to circulating filarial antigen (CFA) status. The treatment regimens were amoxicillin, doxycycline and placebo. Of the 149 treated patients, 51 representing (34.2%) were CFA-positive whereas 98 (65.7%) were CFA- negative. The total number of patients treated with 1000mg/d amoxicillin for 6weeks was 49, out of which 36 completed the treatment with 13 being CFA-positive and 23 CFA-negative. Thirteen patients dropped out (12 due to non-compliance and one was hospitalized for hypertension).

Fifty-four of the lymphedema patients received 200mg/day doxycycline for 6 weeks. Of these, 46 completed the treatment of which 19 were CFA-positive and 27 CFA-negative. Eight (8) patients in this group dropped out (6 due to non-compliance and one with gynecological problem and the remaining (1) was hospitalized as a result of pregnancy issues). The number of lymphedema patients who received placebo for 6 weeks was 46. Patients who completed the treatment in the placebo group were 38 (Fig.1) of which 14 and 24 were CFA- positive and CFA-negative respectively.

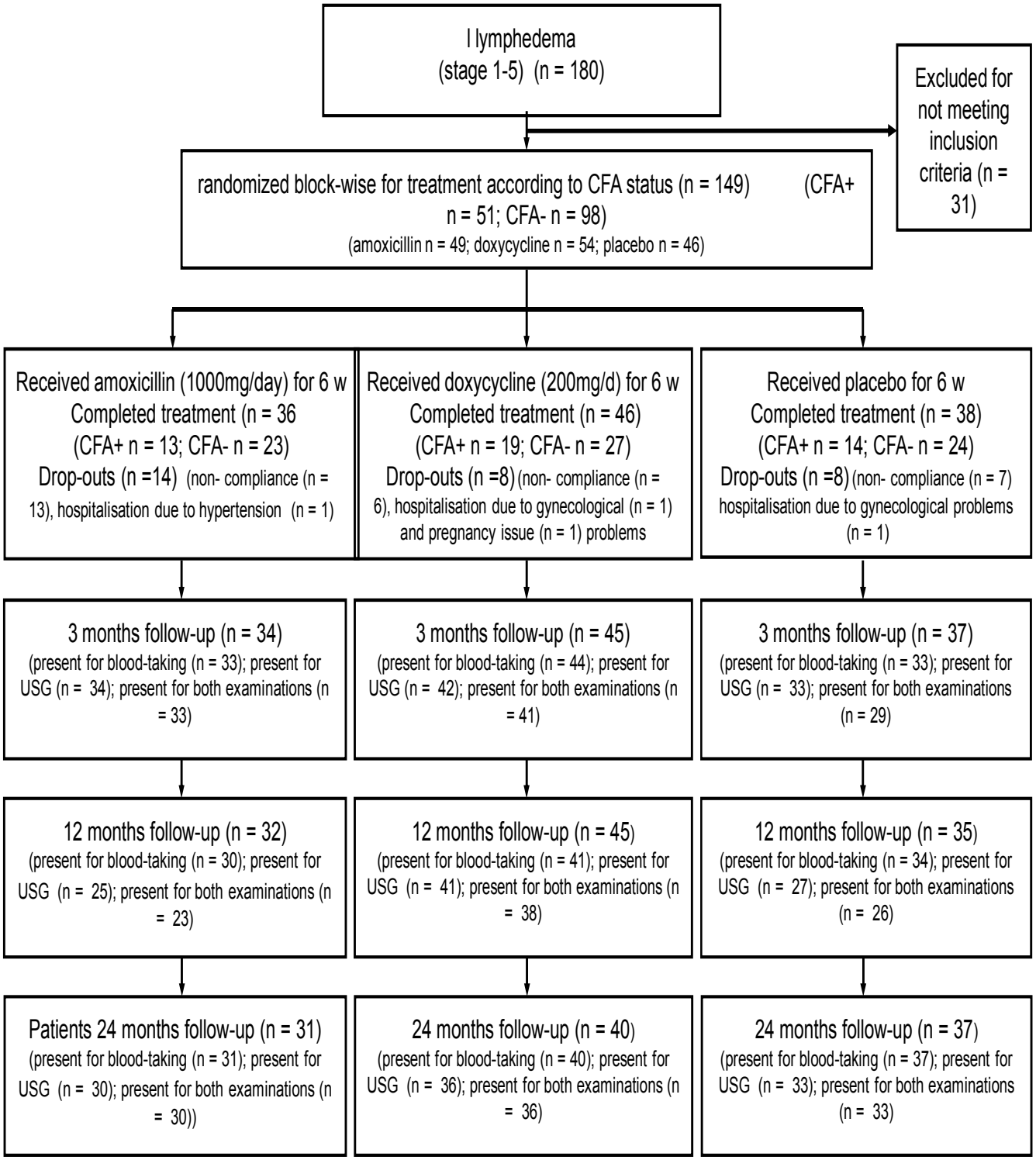


Figure 2.0 Flowchart of the LE patient participation. Of the 180 recruited, 36 treated with amoxicillin, 46 with doxycycline and 38 received placebo for 6 weeks. 117 patients were present at 6months, 112 at the 12 months and 102 present at 24 months time points.

Of the 119 lymphedema patients who successfully completed the treatment, 34 (28.6%) were males whereas 85 (71.4%) were females. There was no significant difference in the randomization of males and females in the various treatment arms (p=0.182, Chi square test). The subjects in this trial had a mean age of 47.7±10.8. Patients who received amoxicillin were significantly older than those who received doxycycline (p=0.03, Mann Whitney) or placebo (p=0.028, Mann-Whitney). There was no significant difference between doxycycline- and placebo -patients regarding age using Mann-Whitney U test. From Table 1 patients who were CFA-positive at the treatment start had a higher mean age than patients who were CFA-negative. Ivermectin (IVM) distribution was done three times during the clinical trial, in 2006, 2007 and 2008 consecutively. There was 74% coverage in the first two years of distribution. During the third IVM distribution the compliance had decreased to 69%, however there was no significant difference in the various treatment regimens in terms of IVM intake (Table 1).

4.2 Adverse effects of doxycycline, amoxicillin and placebo treatment

Most adverse events arose on days 2 and 3 after start of treatment in the three groups; no adverse events took place after 10 days. Doxycycline was well tolerated. The adverse reactions reported after doxycycline (n=8), amoxicillin (n=9) and placebo (n=6) were mild and tolerable. Of individuals who experienced adverse reactions, each reported a single symptom. In the doxycycline group, adverse events included headache, nausea, diarrhea, vomiting, dizziness and pruritus. In the amoxicillin group, reported adverse events were fever, headache, orchitis, and epigastric pain. Similarly, in the placebo group the complaints were stomach pains, headache and dizziness. No evidence of photosensitivity reactions to doxycycline was recorded and no individual had to stop

taking doxycycline because of adverse events. None of the patients was asked to stop treatment because of adverse effects of treatment.

Table 1 **Demographic data and stratifications of various treatment groups**

	Total	**Doxycycline**	**Amoxicillin**	**Placebo**	***p-value***
No. of patients at treatment start	149	54	49	46	
No. of patients who completed treatment	119	46	35	38	
Male	34	10	9	15	0.182*
Female	85	36	26	23	
Age(mean ± SD)[1]	47.7±10.8	46.1±11.6	51.3±9.1	46.3±10.9	0.045#
CFA-positive	49.7±12.1[2]	45.5±13.9	56.0±5.6	49.4±12.1	0.045#
CFA- negative	46.5±9.8[2]	46.5±9.9	48.6±9.7	44.5±9.9	0.304#
Weight (mean±SD)	65.0±15.8	66.8±16.2	62.8±16.7	65.0±14.5	0.389#
LE since(years) (mean±SD)	13.8±12.3	13.7±11.8	14.6±14.9	13.3±10.5	0.893#
CFA- positive	11.5±12.7[3]	9.3±10.2	12.2±16.5	13.7±12.3	0.457#
CFA- negative	15.3±11.9[3]	16.9±11.9	16.0±14.1	13.0±9.5	0.554#
p-value	0.029¶[3]				
IVM 2006/total no.	87/117(74%)	31/46	28/34	28/37	0.304*
IVM 2007/total no.	85/115(74%)	30/44	27/34	28/37	0.512*
IVM 2008/total no.	75/109(69%)	25/41	24/31	26/37	0.317*

***Chi square # Kruskal- Wallis, ¶ Mann- Whitney U**

4.3 Microfilaraemia and Antigenaemia values of *Wuchereria bancrofti* from start of treatment

In the doxycycline group, 6 (13.0%) out of 46 patients who completed treatment were MF- positive at the start of treatment. At 3 months' follow-up, there was 67.7% reduction in microfilaria positive patients in the doxycycline group. At 12 and 24 months follow-ups, there were virtually no MF- positive patients in the doxycycline group suggesting 100% reduction of MF from this group.

The number of MF-positives in the amoxicillin group was 3 out of 35, which also reduced substantially at the 3-month follow- up recording 67.7% reduction in MF-positive subjects. However, during the 12-month follow-up, 4 of these subjects in the

amoxicillin group became MF-positive representing an increase of 133%. Finally, at 24-month follow-up, 2 amoxicillin patients were still positive for MF representing (33.3%) reduction. In the 38 patients who received placebo, the total number of MF-positives was 2 (2.6%). During subsequent follow- ups only 1 was microfilaraemic representing (50%) reduction (Table 2).

The number of patients positive for CFA fell significantly in the doxycycline group from 19 to 13 representing (31.57%) of pretreatment CFA-positives at 12 and 24 months compared to a reduction from 13 to 10 (23%) of pretreatment CFA-positives observed in the amoxicillin group for 12 and 24 months respectively. Interestingly, in the placebo group, filarial antigen positive subjects decreased from 14 to 10 (28.5%) of pretreatment number at 12 months and further decreased to 9 representing (35.7%) at 24 months (Table 2)

Table 2 **Microfilaraemia and antigenaemia levels of *W. bancrofti* at study time points**

Group A (doxycycline 200mg/d)	**Pre-treatment**	**3-month follow-up**	**12-month follow-up**	**24-month follow-up**
Microfilaraemia				
No. of MF- positive/total no. of patients (%)	6/46 (13%)	2/44 (4.5%)	0/41 (0%)	0/39 (0%)
Antigen –status				
No. of CFA – positive/total no. of patients (%)	19/46 (41.3%)		13/41 (31.7%)	13/40(32.5%)
Median (25^{th} -75^{th} percentile)	72 (21-24846)		9.5 (5.8-15177)	7.3 (5.7-2893)
p-value*			0.396	0.004
p-value (CFA- positive pre-treatment)			0.084	0.679
p-value (CFA-negative pre-treatment)			0.001	< 0.001
Group B (amoxicillin 1000mg/d)				
Microfilaremia				
No. of MF- positive/total no. of patients (%)	3/35 (8.6%)	1/33 (3.0%)	4/30(13.3%)	2/31 (6.5%)
Antigen-status				
No. of CFA – positive/total no. of patients (%)	13/35 (37.1%)		10/30 (33.3%)	10/30 (33.3%)
Median (25^{th} -75^{th} percentile)	104.5 (21-25688)		7.7 (5.6-25710)	7.5 (5.4-19389)
p-value*			0.673	0.045
p-value (CFA- positive pre-treatment)			0.11	0.859
p-value (CFA-negative pre-treatment)			0.005	0.003
Group C (placebo)				
Microfilaremia				
No. of MF- positive/total no. of patients (%)	2/38 (5.3%)	1/34 (2.9%)	1/34 (2.9%)	1/37 (2.7%)
Antigen-status				
No. of CFA – positive/total no. of patients (%)	14/38 (36.8%)		10/33 (30.3%)	9/36 (25.0%)
Median (25^{th} -75^{th} percentile)	52.5 (28-7179)		8.9 (5.7-1614)	6.3 (5.3-513)
p-value*			0.036	0.003
p-value (CFA- positive pre-treatment)			0.695	0.507
p-value (CFA-negative pre-treatment)			< 0.001	<0.001

The median values of antigen before treatment was 72 in the doxycycline group, 104.5 in the amoxicillin group while 52.5 recorded for the placebo group. The values fell substantially in all the groups at 12 and 24 months start of treatment (Table 2). There was no significant difference in CFA-positives in the doxycycline, amoxicillin and placebo groups comparing pretreatment and 12 months post-treatment regarding antigenaemia levels (p=0.084, p=0.11, p=0.695 respectively, Wilcoxon Rank test). A similar observation was made comparing pretreatment and 24 months post-treatment in all the treatment arms for filarial antigens levels as presented in (Table 2).

4.4 Lymphedema staging- sum of both legs

There was no difference between the three groups regarding pretreatment staging in both CFA+ and CFA- patients (p=0.239, Kruskal-Wallis) (Table 2). The mean leg stage for patients treated with doxycycline decreased consistently at 3 months and progressively through 12 months to 24 months (p=0.527, p=0.421, p=0.023 respectively) (Table 3)

There was no significant difference in the mean leg stage in the CFA-positive subjects in the doxycycline group comparing pretreatment and follow-ups using Wilcoxon paired test as indicated in (Table 3). However, in the CFA-negative patients, there was significant difference in the mean leg stage comparing pretreatment and 24 months post-treatment (p=0.022, Wilcoxon test) (Table 3). In the amoxicillin group, though there was no significant difference in mean leg stage at 3 and 12 months, there was remarkable difference towards deterioration at 24 months post-treatment (p=0.005, Wilcoxon test) (Table 3)

In patients who received placebo, mean leg stage remained steady during the 3 months ($p=0.206$, Wilcoxon test) but increased during the 12months follow up ($p=0.02$, Wilcoxon test). At 24 months follow-up, the mean of the leg stage significantly increased ($p<0.001$, Wilcoxon test). In the CFA-positive placebo-treated patients, though the mean leg stage increased across the treatment time points it did not result in any statistically significant difference. On the other hand with the CFA-negative patients the mean leg stage increased consistently in this group resulting in significant difference as shown in (Table 3)

Table 3 **The mean and median of lymphedema staging of sum of both legs**

Group A (doxycycline)	**Pre-treatment**	**3-month follow-up**	**12-month follow-up**	**24-month follow-up**
N	46	44	45	41
Mean ±SD (range)	3.8 ±2.1(1-9)	3.6±2.1(1-9)	3.7±2.7(0-12)	3.3±2.6(0-9)
Median (25^{th} -75^{th} percentile)	3(2;5.3)	3(2;5)	3(2;5)	3(1;5)
p-value*		0.527	0.421	0.023
CFA-positive				
N	19	19	19	17
Mean ±SD (range)	3.4±2.1(1-9)	3.5±2.3(1-9)	3.6±3.0(0-12)	3.2±2.7(0-9)
Median (25^{th} -75^{th} percentile)	3(2;4)	3(2;4)	3(2;4)	3(1;4)
p-value*		0.317	0.719	0.454
CFA-negative				
N	27	25	26	24
Mean ±SD (range)	4.0±2.1(2-9)	3.6±1.9(1-8)	3.7±2.6(0-9)	3.5±2.5(0-9)
Median (25^{th} -75^{th} percentile)	3(2;6)	3(2;5.5)	3(2;5.3)	3(1.3;5)
p-value*		0.102	0.184	0.022
Group B(amoxicillin)				
N	35	34	31	31
Mean ±SD (range)	3.8±2.4(1-9)	3.9±2.4(1-9)	4.3±2.8(1-12)	4.5±3.1(1-12)
Median (25^{th} -75^{th} percentile)	3(2;6)	3(2;6)	3(2;6)	4(2;6)
p-value*		0.334	0.223	0.005
CFA-positive				
N	13	13	11	12
Mean ±SD (range)	3.3±1.9(1-6)	3.5±1.9(1-6)	3.4±1.9(1-6)	3.8±2.3(1-8)
Median (25^{th} -75^{th} percentile)	2(2;6)	3(2;6)	3(2;6)	3(2;6)
p-value*		0.317	0.414	0.102
CFA-negative				
N	22	21	20	19
Mean ±SD (range)	4.1±2.6(2-9)	4.2±2.7(1-9)	4.8±3.2(1-12)	5±3.5(1-12)
Median (25^{th} -75^{th} percentile)	3(2;5.3)	3(2;6)	4(2;7.5)	4(2;8)
p-value*		0.705	0.054	0.019
Group C(placebo)				
N	38	36	34	36
Mean±SD (range)	4.6±2.7(2-11)	4.6±2.9(1-11)	5.3±3.5(0-12)	5.5±3.4(0-12)
Median (25^{th} -75^{th} percentile)	3(2.8;6)	3(2;6)	3.5(2.8;9)	5(3;6)
p-value*		0.06	0.02	<0.001
CFA-positive				
N	14	13	11	13
Mean±SD (range)	4.7±3.0(2-11)	4.8±3.1(2-11)	5.8±4.0(0-12)	6.0±3.7(0-12)
Median (25^{th} -75^{th} percentile)	4(2.8;5.3)	4(2.5;6)	6(3;11)	6(3;8.5)
p-value*		0.317	0.144	0.065
CFA-negative N	24	23	23	23
Mean ±SD(range)	4.5±2.7(2-9)	4.5±2.9(1-10)	5.0±3.3(2-12)	5.2±3.0(2-12)
Median (25^{th} -75^{th} percentile)	3(2.3;6)	3(2;7)	3(2;9)	4(3;6)
p-value*		0.317	0.046	0.001

From table 4.0 there was no difference between pretreatment and 3-month follow-up regarding all the three groups (p=0.407), CFA-positive (p=0.96.7) and CFA-negative (p=0.197) when Kuskal-Wallis test was used. In a similar comparison between pretreatment and 12-month follow-up, there was significant difference among all the three treatment groups. There was no significant difference associated with the CFA-positive (p=0.487) while CFA-negative group recorded a significant difference (p=0.005).

Table 4 **Difference within treatment groups of LE staging at various time points**

	Difference: 3 –month follow-up -pretreatment	**Difference: 12-month follow-up -pretreatment**	**Difference: 24-month follow-up -pretreatment**
Comparison of all 3 groups#	*p*=0.407	*p*=0.019	*p*<0.001
CFA -positive	*p*=0.967	*p*=0.487	*p*=0.037
CFA-negative	*p*=0.197	*p*=0.005	*p*<0.001
Doxycycline vs. Amoxicillin¶	nd	*p*=0.011	*p*<0.001
CFA -positive	nd	nd	*p*=0.025
CFA-negative	nd	*p*=0.007	*p*<0.001
Doxycycline vs. Placebo¶	nd	*p*=0.05	*p*<0.001
CFA-positive	nd	nd	*p*=0.086
CFA- negative	nd	*p*=0.006	*p*<0.001
Amoxicillin vs. Placebo¶	nd	*p*=0.455	*p*=0.121
CFA-positive	nd	nd	*p*=0.216
CFA- negative	nd	*p*=0.944	*p*=0.391

nd= no difference, since there was no significant difference comparing all 3 groups, # Kruskal-Wallis,¶ Mann-Whitney U.

Between pretreatment and 24 months follow–up, there was significant difference among the treatment groups in both CFA-positive and CFA-negative groups. There was also significant difference between the doxycycline and amoxicillin in both CFA-positive and CFA-negative groups as indicated in (Table 4). A similar pattern described above was

observed between the doxycycline and the placebo groups, as shown by their CFA-negatives whereas their CFA-positives showed not statistically significant difference. Comparing amoxicillin and placebo groups there was significant difference as shown in (Table 4)

4.4.1 Assessment of lymphedema staging on improvement, halt of progression and pathology progression at 24 months after treatment

Table 5 **Performance of treatment on improvement, halt of progression and deterioration at 24 months**

	Improvement	**Halt of progression**	**Deterioration**
Doxycycline	18/41(43.9%)	18/41(43.9%)	5/41(12.2%)
Amoxicillin	1/31 (3.2%)	19/31 (61.3%)	11/31 (35.5%)
Placebo	2/36 (5.6%)	12/36 (33.3%)	22/36 (61.1%)
p=value*	p<0.001		

*Pearson's chi squared-test

Assessment on patients' lymphedema staging was compared in the three treatment arms. In the doxycycline group, out of 41 subjects, 18(43.9%) had improvement in leg staging which was considerably significant than the amoxicillin group where 1 out of 31 representing (3.2%) had improvement in the LE stage. In the placebo group, 2 patients out of 36 representing (5.6%) had improved condition in the LE stage as shown in (Table 5).

Halt of progression of lymphedema stage was seen in 61.3% of the amoxicillin treated group followed by doxycycline and placebo groups where 43.9% and 33.3% were obtained respectively (Table 5).

Five (5) out of 41 representing (12.2%) in the doxycycline group had deteriorated lymphedema stage followed by 11out of 31(35.5%) in the amoxicillin- treated group. In the placebo group, 22 out of 36 representing (61.1%) of the patients had worsened

lymphedema stage. Pearson's square test, showed significant difference in lymphedema stage regarding the number of patients in the various treatment arms that had improvement, halt of progression and deterioration 24 months after treatment (Table 5)

4.5 Circumferential measurement of the lymphedema legs

Measurement of the leg circumference is one of the methods used to determine an increase or decrease in leg volume at stipulated positions at treatment time points as stated in the Materials and Methods. Circumference measurement was done for all the recruited patients throughout the study time points. This is consistent with protocols set by Dreyer *et al.,* (2002).

Measured at:

-10cm (from big toe towards Mall. med.)

-12cm (from the heel upwards)

-20cm (from the heel upwards)

-30cm (from the heel upwards)

-50 or 60 cm (from the heel upwards, above the knee)

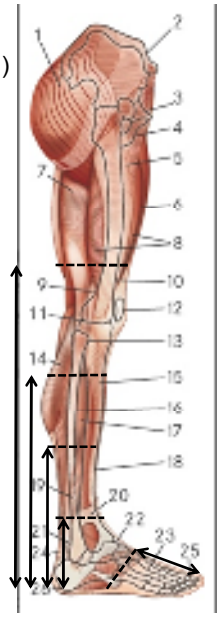


Figure 3.0 Circumferential measurements at various positions of the leg. Measurement done at 10cm, 12cm, 20cm, 30, 50cm or 60cm in lymphedema patients at pre, 3 months,12months and 24 months post treatment in accordance with protocol established by Dreyer *et al.,* (2002) www.wilkapedia.org

Table 6 **Circumference measurement of sum of both legs 10-30cm**

Group A (Doxycycline)	**Pre-treatment**	**3 –month follow-up**	**12-month follow-up**	**24-month follow-up**
N	43	40	41	39
Mean ±SD	221.3±27.6	223.1±31	223.7±33.4	221±24.2
Range	178.5-299	177.5-302.3	177.2-330.3	177.8-338.4
Median (25^{th} -75^{th} percentile)	216(200;233)	214.4(201.9; 238.4)	216.3(199.7;239.7)	212(196.5;232.3)
p-value*		0.322	0.538	0.03
Group B (Amoxicillin)				
N	33	34	32	30
Mean ±SD	217.3 ±29.4	220.8±34.4	220±36.2	218.3±34.8
Range	171-289	171.2-320	172.2-326	167.6-333.8
Median (25^{th} -75^{th} percentile)	217(195;230.5)	217.8(2193.8; 236.6)	213.4(193;240.8)	215.9(191.3;234.6)
p-value		0.623	0.213	0.206
Group C (Placebo)				
N	33	33	30	32
Mean ±SD	225.2±29.4	226.4±27.2	226.34.3	218.3±36.8
Range	167-302	171.1-296.9	165.2-312	168.9-311.7
Median (25^{th} -75^{th} percentile)	225(203.8;244)	230(206.7-243.3)	232.3(201;243.3)	227.8(201.8;243.1)
p-value		0.077	0.059	0.171

*Wilcoxon pre-treatment vs. follow-up, No difference between the 3 groups regarding circumference measurement pre-treatment p=0.351 (Kruskal-Wallis). SD=Standard Deviation

4.5.1 Effects of treatment on mean of sum of leg circumference measurement of both legs (10-30cm) comparing pretreatment versus various time points

There was no difference between the three groups regarding mean leg measurement at pre-treatement (p=0.351, Kruskal-Wallis).

The mean of leg masurements of the patients in the doxycycline group increased slightly at both 3-and 12-months (Table 6), however at 24-months there was a reduction in the mean measurement (p=0.03) (Table 6).

In the amoxicilllin group, there was no significant difference comparing pretreatment to all the time-points. Although there was a slight decrease at 24 -months' there was no significant difference (p=0.206). Comparing mean leg measurements in the placebo

group, there was no significant change at 3-month and 12-month follow-ups, however , at 24-months the mean had decreased to almost pretreatment level (Table 6).

4.6 Malleolus lateralis and medialis measurements

The thickness of the skin at the ankle was measured at all time points using ultrasound machine.

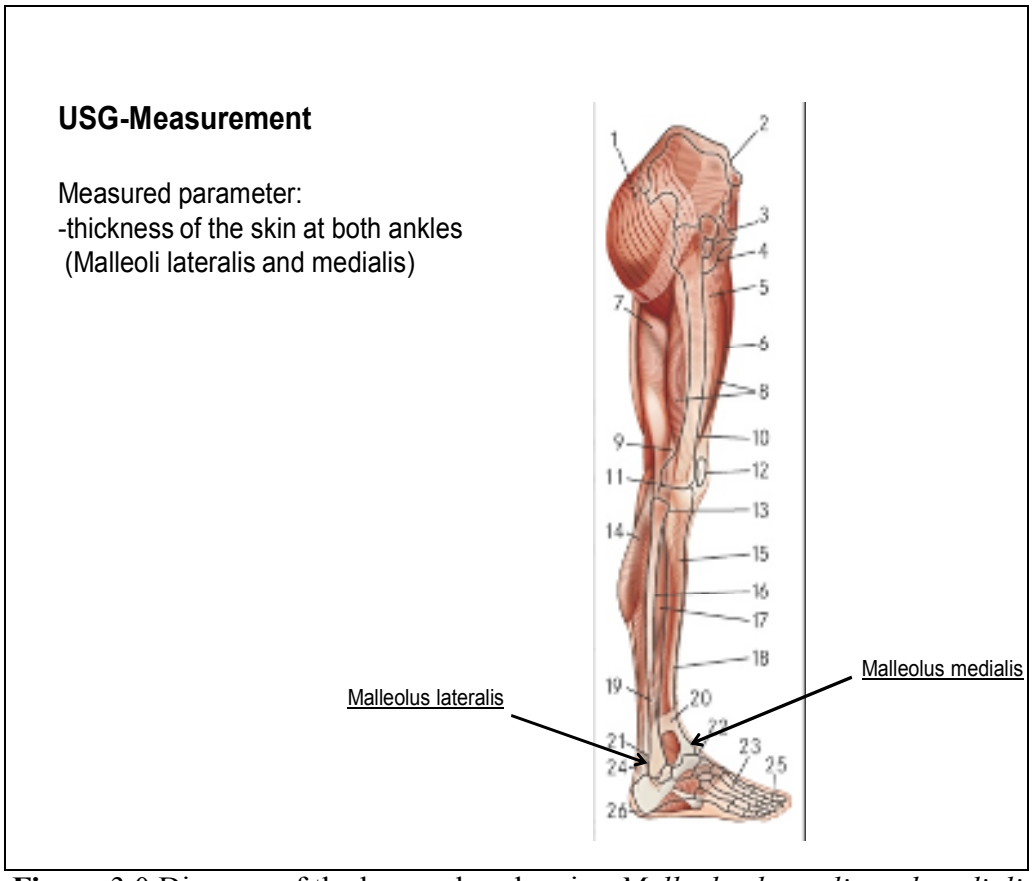


Figure 3.0 Diagram of the human leg showing *Malleolus lateralis and medialis* **www.wilkapedia.org**

Table 7 **Malleolus measurement** of both legs in treatment arms at various time points

Group A (doxycycline 200mg/d)	**Pre-treatment**	**3 –month follow-up**	**12-month follow-up**	**24-month follow-up**
N	42	42	41	37
Mean ±SD	0.74 ±0.44	0.65±0.4	0.64±0.41	0.61±0.42
Range	0.29-2.07	0.26-2.28	0.23-1.91	0.21-2.14
Median (25^{th} -75^{th} percentile)	0.6(0.5;1.0)	0.5(0.4;0.8)	0.5(0.3;0.9)	0.5(0.3;0.7)
p-value*		0.009	<0.001	0.001
Group B(amoxicillin 1000mg/d)				
N	32	34	25	30
Mean ±SD	0.68±0.4	0.65±0.39	0.68±0.47	0.73±0.51
Range	0.28-1.62	0.23-1.62	0.21-1.77	0.26-2.32
Median (25^{th} -75^{th} percentile)	0.5(0.4;0.9)	0.5(0.3;0.8)	0.4(0.4;0.8)	0.6(0.4;1.0)
p-value*		0.03	0.745	0.716
Group C(placebo)				
N	33	33	26	32
Mean±SD	0.78±0.39	0.74±0.47	0.76±0.48	0.8±0.4
Range	0.32-1.75	0.28-2.01	0.3-1.93	0.26-1.8
Median (25^{th} -75^{th} percentile)	0.7(0.5;1.0)	0.6(0.4;0.8)	0.6(0.4;0.9)	0.7(0.5;1.0)
p-value*		0.34	0.493	0.06

***Wilcoxon pretreatment vs. follow-up, SD= Standard deviation**

4.6.1 Effects of treatment on malleolus measurement at various time points

There was significant difference in skin thickness measurements between doxycycline versus amoxicillin, and also between doxycycline and placebo but no significant difference between amoxicillin and placebo especially at 12 and 24 months after start of treatment (Table 7)

Table 8 **The Geometric mean of the sum of *Malleolus lateralis* of both legs staging stratified according to CFA status at various time points.**

Treatment Group	***Malleolus lateralis* sum of both legs**	
	Geometric Mean	
	CFA positive	CFA negative
6 weeks Doxycycline (n=CFA+15,CFA-19)		
Pre- treat	1.07	1.13
3 mo	1.1	0.99
12 mo	1.05	0.89
24 mo	1.0	0.83
p value (Friedman)	**0.1**	**0.0001**
6 weeks Amoxicillin (n=CFA+ 8, CFA-13)		
Pre- treat	0.88	1.2
3 mo	0.97	1.12
12 mo	0.83	1.16
24mo	1.0	1.19
p value(Friedman)	**0.005**	**0.52**
6 weeks Placebo (n=CFA+18,CFA-16)		
Pre- treat	1.21	1.21
3 mo	1.15	1.13
12mo	1.19	1.15
24 mo	1.29	1.25
p value (Friedman)	**0.37**	**0.35**

4.6.2 Efficacy of treatment on mean of the sum of *Malleolus lateralis* of both legs split by CFA status at various time points.

In the CFA-positive amoxicillin-treated patients, the pretreatment mean recorded increased at 3 months but reduced in a year later (12 months). The mean finally increased slightly at 24 months follow-up. There was a highly significant difference in the CFA-positives under this group (p=0.005, Friedman test) towards increase in skin thickness.

Similarly, in the CFA-negative patients, there was a consistent increase in the mean to the end of the study but there was no significant difference (p=0.52) (Table 8)

In doxycycline-treated CFA-positive patients, there was a consistent decrease in the mean at 3 months and continued to decline at 24 months post treatment. However, no significant difference was obtained (p=0.1, Friedman test). Interestingly, in CFA-negative patients there was a drastic significant decrease in the skin thickness measured at the *Malleolus lateralis* for all follow -up time points (p=0.0001, Friedman test).

CFA-positive patients who received placebo showed a reduction in the mean from pretreatment through 3 months but increased at 12 months and 24 months (Table 8). In the CFA-negative placebo-treated patients, a similar trend was recorded as in the CFA positive-patients. There was no significant difference in the two groups (CFA+ and CFA- p=0.37, p=0.35 respectively) (Table 8)

Table 9 **The Geometric mean of the sum of *Malleolus medialis* of both legs stratified by CFA status at various time points**

Treatment Group	***Malleolus medialis*- sum of both legs**	
	Geometric mean	
	CFA positive	CFA negative
6 weeks Doxycycline (n=CFA+15,CFA-19)		
Pre- treat	1.18	1.46
3 mo	1.19	1.30
12 mo	1.17	1.17
24 mo	1.18	1.16
p value (Friedman)	**0.71**	**0.0001**
6 weeks Amoxicillin (n=CFA+ 8, CFA-13)		
Pre- treat	1.09	1.52
3 mo	1.19	1.42
12 mo	1.00	1.52
24mo	1.27	1.54
p value(Friedman)	**0.02**	**0.06**
6 weeks Placebo (n=CFA+8,CFA-16)		
Pre- treat	1.44	1.53
3 mo	1.30	1.37
12mo	1.27	1.43
24 mo	1.50	1.67
p value (Friedman)	**0.14**	**0.015**

4.6.3 Efficacy of treatment on mean of sum of *Malleolus medialis* of both legs staging according to CFA status at study time points

The trend of mean skin thickness at the malleolus medialis in the various treatment arms at study time points is shown in (Table 9). In the amoxicillin group, the mean of malleolus medialis of CFA-positive patients increased significantly from pretreatment throughout to 24months time points (p=0.02). In the CFA negative amoxicillin-treated

patients, the malleolus medialis reduced at 3 months but increased at 12 and 24 months (p=0.06) (Table 9).

In the CFA-positive patients treated with doxycycline, malleolus medialis increased at 3 months follow-up but decreased during 12 months reexamination and finally increased again at 24 months (p=0.71) (Table 9) whereas in the CFA-negative patients treated with doxycycline, there were significant reduction of malleolus medialis at all follow-up times (p=0.0001) (Table 9)

From table 9, CFA-positive patients who received placebo had a decrease in malleolus medialis at 3 and 12 months. However, at 24 months, the malleolus medialis had increased notably (Table 9). In the CFA-negative placebo-treated patients, the malleolus medialis decreased significantly at 3 months but increased at 12 months and 24 months (p=0.015) (Table 9)

Table 10 **Incidence of acute attacks during the study period**

	Appearance of "acute attacks"	
	No	Yes
Doxycycline	19/41 (46.3%)	22/41 (53.7%)
Amoxicillin	10/31 (32.3%)	21/31 (67.7%)
Placebo	6/36 (16.7%)	30/36 (83.3%)
p=value*	p=0.021	
Doxycycline vs. Placebo: p=0.006* Doxycycline vs. Amoxicillin:p=0.228* Amoxicillin vs. Placebo: p=0.136*		

*Pearson's chi square

4.7 Incidence of acute attacks during study period

Attack was only considered as real attack if patient confirmed the presence of fever, lymph node swelling (inguinal or femoral), pains and sometimes peeling of affected leg(s).

Nineteen (19) doxycycline-treated patients out of 41 (46.3%) had no filarial attacks during the 24 months follow-up whereas 22 out of 41(53.7%) had acute filarial attacks. In the amoxicillin-treated patients, 32.3% had no filarial attacks while 67.7% had attacks at the end of 24 months post-treatment (Table 10). In the placebo group, 16.7% had no filarial attacks at 24 months while 83.3% experienced acute filarial attacks. Pearson's chi square showed significant difference among the three treatment groups on incidence of acute attacks (Table 10). Doxycycline best reduces filarial attacks than amoxicillin and placebo respectively (Table 10)

CHAPTER 5

5.0 DISCUSSION

5.1 Anti-wolbachial treatment in lymphedema

This study was carried out to determine the effects of treatment in lymphedema subjects grouped under circulating filarial antigens status (with or without active infection) comparing 200mg/d of doxycycline an anti-wolbachial agent with 1000mg/d of amoxicillin an antibacterial in a six-week double-blind, randomised placebo-controlled trial.

Globally, over 16 million people are affected with filarial lymphedema (Michael *et al.,* 1996). Lymphedema is known to present untold economic hardship through reduced existing workforce of affected endemic communities (Ramaiah *et al.,* 2000). Currently, there are no clear treatment modalities for lymphedema because of inadequate information regarding the mechanisms of pathogenesis. Several studies, however, recommend the use of antibiotics since recurrent acute attacks in lymphedema are believed to be caused by secondary bacterial infections (Casley- Smith *et al.,* 1993; Pani *et al.,* 1989).

There are evidences that diethylcarbamazine (DEC) treatment alone does not have a significant change either in lymphedema volume (Das *et al.,* 2003) or lymphatic pathology (Freedman *et al.,* 1995). Other drugs prescribed by clinicians are generally anti-helminthics such as ivermectin, albendazole and at times diuretics. Although ivermectin is an effective microfilaricidal agent, currently there is no evidence of its role for treating lymphedema (Dreyer *et al.,* 1996; Shenoy *et al.,* 1998). According to Ottesen

and colleagues (1999) the role of albendazole is also not clear in the management of LF morbidity. Regarding the use of diuretics, different opinions are expressed. Though these drugs are used for treating non-filarial edema, they are not effective in filarial edema (McGuinness and Burnand, 2001). So far other chemotherapeutic agents investigated such as 5, 6, benzo-alpha-pyrone (coumarin) has shown promising results. This was demonstrated in studies on bancroftian lymphedema cases in South India and China, where coumarin significantly reduced edema volume (Casley-Smith *et al.* 1993) but unfortunately this drug cannot be used because of its associated hepatoxicity (WHO, 1996).

Zelikovski and colleagues (1993) have also suggested that physiotherapeutic measures such as pneumatic compression, manual massage and interferential current therapy prove useful in edema volume reduction in non-filarial cases. However, their role is yet to be evaluated in filarial lymphedema. Although Binoy *et al* (1998) proposed surgical procedures to be useful in correcting filarial lymphedema, only a few specialized centres exist. This makes the surgical based approach unsustainable.

The tremendous success achieved by doxycycline in onchocerciasis and lymphatic filariasis field studies through depletion of endobacteria *Wolbachia* has brought hope to subjects who live in the affected endemic communities (Hoerauf *et al.,* 1999; Taylor *et al.,* 2005). In 2006, Debrah *et al* reported that doxycycline improved filarial lymphedema from a pilot study in the Nzema East District of the Western Region. Therefore there was

the need to replicate this study on larger scale to clearly define the role of doxycycline in filarial lymphedema.

The rationale for including penicillin based therapy (amoxicillin) is due to its proven activities on exogenous bacterial infection in LE (Shenoy *et al.,* 1999) and again since it can be given to children, thus closing one of the gaps that are left by doxycycline.

Anti-wolbachial drugs such as doxycycline and rifampicin are already available in many endemic areas, and have well-established pharmacological, pharmacokinetic and safety information. The principle of anti-wolbachial chemotherapy on filariasis is based on findings in animal models and human studies in lymphatic filariasis and onchocerciasis. The effectiveness of these drugs are seen in the depletion of *Wolbachia* (Hoerauf *et al.,* 2000; 2002; 2003; 2008; Taylor *et al.,* 2005; Tunner *et al.,* 2010) as well as reduction of plasma VEGF which led to improvement in both lymphedema and hydrocele patients (Debrah *et al*., 2006b; 2007a; 2009).

From this study, it is obvious that the report by Debrah *et al* (2006) where the depletion of *Wolbachia* resulted in reduced plasma VEGFs and finally led to improved pathology in lymphatic filariasis is apparently not the only mechanistic pathway used by doxycycline to achieve the led improvement particularly considering those without active infection. Interestingly, a similar study carried out in the northern part of Cameroon, six weeks doxycycline treatment of podoconiosis-a non filarial lymphedema resulted in a remarkable improvement in pathology even in the absence of *Wolbachia* (unpublished data, personal communication). As a bacteriostatic and a broad spectrum agent; doxycycline is highly effective against many microorganisms, including *Streptococcus*

pyogenes and *Staphylococcus aureus* (Krakauer *et al.,* 2003) which contribute to the pathogenesis LE. Current studies indicate that, doxycycline has considerable non-antimicrobial effects on the host as seen in cases of cancer metastasis, periodontitis, autoimmune disorders, rheumatoid arthritis and scleroderma (Sapadin *et al.,* 2006; Krakauer *et al.,* 2003) and anti-oxidative stress (Soory, 2008).

5.2 Microfilariae and Antigenaemia

5.2.1 Effect of treatment on microfilariae and antigenaemia levels

There was 100% reduction in the number of LE microfilaria positive patients who received 6 weeks of doxycycline treatment at the end of the study (Table 2). This finding is consistent with earlier reports from Tanzania by Taylor and colleagues (2005) as well as by Debrah *et al* (2006b, 2007b) from Ghana where 8-and 6-week courses of doxycycline respectively, resulted in complete elimination of microfilariae. Similarly, the drastic reduction of microfilariae in the doxycycline group at 24months confirms the prolonged sterilizing and embryostatic effects of doxycycline on the adult worm reproductive potential (Hoerauf *et al.,* 2000; 2001; 2003; 2008; Turner *et al.,* 2010).

However, in the amoxicillin-treated patients as well as the placebo group, some patients still harbored microfilaria at 24 months after start of treatment suggesting amoxicillin has no microfilaricidal and anti-wolbachial effects. Therefore in seeking for a suitable drug for lymphedema treatment with micro- and macrofilaricidal effects, amoxicillin comes second to doxycycline since it target only exogenous bacterial. Furthermore, amoxicillin

has no known effects on any of the stages of the filarial worm and *Wolbachia* endosymbiont bacterial known to play essential role in the pathogenesis of LE. Although neither amoxicillin nor placebo has microfilaricidal effects, there was slight reduction in the number of mf positive patients in these two groups after the study period (Table 2). The marginal reduction of microfilarial levels in both amoxicillin and placebo was due to the thrice ivermectin distribution.

Antigenaemia levels also decreased significantly at 24months in the doxycycline-treated subjects compared to pretreatment values (Table 2). The reduction in antigenaemia levels confirmed the macrofilaricidal effects of doxycycline against *Wuchereria bancrofti* as already reported by (Taylor *et al.,* 2005; Debrah *et al.,* 2006b) and also indicated recently in *Onchocerca volvulus* (Hoerauf *et al.,* 2008) as well as *Onchocerca volvulus* co-infected *with Loa loa* in Cameroon (Turner *et al*., 2010).

The few doxycycline-treated patients who were still positive for circulating filarial antigens at end of the study confirms earlier report (Debrah *et al*. 2006b) where a similar trend was seen. This result clearly espouses the idea that the slow excretion rate of antigen from the human body after death of adult worms is likely to occur particularly in those with higher worm burden. Therefore it can be circumstantially hypothesized that, complete elimination of filarial antigens takes a little over 24 months which agrees with studies by Eberhard *et al.* (1997) where they concluded that although filarial antigen fell after treatment, in no case did it fall to zero, even in individuals who remained amicrofilaraemic for several years after treatment, suggesting that some adult worms survived.

Moreover, the less significantly reduced antigenaemia levels measured in the amoxicillin and placebo groups could be due to natural attrition of the adult worms since neither amoxicillin nor placebo has adulticidal effects.

5.3 Lymphedema staging

5.3.1 Effect of treatment on the stage of the legs

There was significant ameliorations of the stage of the leg in the doxycycline group ($p=0.023$). The leg stage in amoxicillin and placebo groups deteriorated significantly (Table 3) during the two-year study. Improvement of leg stage in the doxycycline-treated patients manifested as better skin texture, few mossy lesions, reduced knobs, fewer deep folds, and also fewer entry lesions of the affected legs. The pattern is consistent with earlier findings by Debrah and colleagues (2006b) when a 6-week course of doxycycline in a pilot study reversed early-stage lymphedema and halted disease progression.

The ameliorative effects seen in the leg stages in the majority of doxycycline-treated patients could be as a result of the drug's anti-inflammatory (Krakauer *et al.,* 2003; Sapadin *et al.,* 2006), antimicrobial properties (Sapadin *et al.,* 2006; Krakauer *et al.,* 2003), activity on plasma VEGF (Debrah *et al.,* 2006b; 2007a) and anti-oxidative stress effects (Soory, 2008) as reported by other independent investigators since almost 60% of these patients were without active infection.

Surprisingly, no improvement was seen in the doxycycline-treated CFA-positives at all follow-up time points (p=0.454). The pattern observed was unexpected, because with the presence of *Wolbachia,* one expected the effect of doxycycline in the CFA-positive to be stronger, since the depletion of *Wolbachia* led to reduced proinflammatory responses (Debrah *et al.,* 2006b). This finding contradicts previous publication by Debrah and colleagues (2006b) which reported significant improvement in most of the LE CFA-positive patients who received doxycycline treatment after 12 months assessment. However, it is possible small sample size and the greater number of patients with higher leg stage might have contributed to the difficulty in detecting small significant difference in the CFA-positive group in this study.

The remarkable improvement in the leg stage of the CFA-negative doxycycline-treated patients cannot be attributed to *Wolbachia* depletion as suggested by (Debrah *et al.,* 2006b) since almost 60% of the doxycycline-treated patients had no active infection. This finding suggests that other possible activities of doxycycline exist. Indeed several reports implicate cytokines and chemokines production during the inflammatory state of the pathology, it is believed the anti-inflammatory, antimicrobial, anti-VEGF and anti-oxidative stress properties of doxycycline (Sapadin *et al.,* 2006; Krakauer *et al.*2003; Debrah *et al.,* 2006b; 2007a; Soory 2008) together contribute to the amelioration in the CFA-negative group. These combined activities of doxycycline are entirely different from its direct effects on *Wolbachial* in principle.

Regarding the effect of doxycycline on mean leg stage, there were no improved conditions in some patients in the CFA- positive group at 24 months in contrast with previous reports by Debrah *et al.,* (2006b) where ameliorative effects was reported after

12 months pos-treatment. However, in the CFA-negative patients treated with doxycycline, there was highly significant improvement in leg stage conditions at 24 months after treatment. This was seen as regressed leg stages particularly the early stages, healed sores, fewer mossy and entry lesions, reduced knobs, general ameliorations in skin integrity in this group.

Recently several studies have indicated other mechanisms of action of doxycycline such as its effects on lymphatic vessels (Fainaru *et al.,* 2008b) and anti-oxidative stress (Soory, 2008) leading to improved lymph flow in these patients. Indeed, sealing of lymph vessels and persistent blocking of endothelial proliferation by doxycycline might possibly contribute to reduced extravasations of lymph fluid and vascular permeability of the lymphatic bed as well as reduction in VEGFs levels (Fainaru *et al.,* 2008a; Debrah *et al*., 2006b; 2007a) all probably resulted in the improved leg stage of the doxycycline-treated group. Nevertheless, difference in the effects of doxycycline treatment observed between the CFA-positives and the CFA-negatives could also have host genetic basis. There is an ongoing study looking at the immunogenetics of these groups with LE after doxycycline treatment.

Unimproved LE leg stage conditions in the amoxicillin group contrasts previous report by Shenoy (1999), where improved legs stage was observed after the administration of oral penicillin, a drug with similar properties to amoxicillin against brugian lymphedema. In this current study, worsened leg stage was rather observed in both amoxicillin and placebo groups where most leg stages moved from a lower stage to higher stages with corresponding increase in folds, mossy lesions and more entry lesions.

The persistent deteriorated conditions observed in majority of the amoxicillin and placebo-treated groups also contrast earlier report (Joseph *et al.,* 2004) where improvement was seen in most penicillin-treated subjects. It is obvious exogenous bacteria take advantage of the already comprised skin through entry lesions such as small sores, cuts, ulcers, cracks in lymphedema patients which normally go unnoticed in healthy individuals causing worsened leg stage condition (Joseph *et al.*, 2004) and several investigators have suggested penicillin based therapy for treating LE (Shenoy *et al.,* 1999; Joseph *et al.,* 2004) in the absence of any anti-LE drug.

However, according to the current study most patients treated with amoxicillin and placebo had worsened leg stage after two years. Although, a few had improved conditions which can be attributed to the drugs antimicrobial effects in the case of amoxicillin and placebo groups probably because of proper foot-care hygiene practices. There was no significant difference between the two groups (Amoxicillin and placebo).

In the CFA-positive and CFA-negative patients treated with amoxicillin, there was progressive deterioration of the disease manifestations in some within these groups at study time points. Some of the leg stage increased from lower to higher stages in contrast to several reports by (Shenoy *et al.,* 1998 and 1999). It is possible the initiated inflammatory processes goes on unabated and cannot be down-regulated by amoxicillin since it has no known anti-inflammatory action. It must be stated that in a number of these patients there was halt of progression of the pathology.

Apparently, worsened conditions were confirmed with increases in mossy lesions, sores, cracks, shallow folds becoming deep folds, thickened skin, newly developed and increased number of knobs and ulcers within the two years of the clinical trial. It must be fairly established that there were few patients of both CFA-positive and negative status that had improved conditions corresponding with head sores, ulcers and improved skin texture particularly at 12 months post-treatment.

In a number of the amoxicillin treated patients there was no improvement in the leg stage for the CFA-positive and CFA-negatives comparing pretreatment stage with 24 months after treatment. It is clear that in a majority patients treated with amoxicillin there was halt of progression of pathology.Amoxicillin could not effect significant improvement in terms of regressing LE stage which is consistent with work by Joseph *et al* (2004) but in contrast to earlier reports by (Shenoy *et al.,* 1998; 1999) where regression of leg stage was seen in most patients after oral penicillin treatment. The deterioration this group possibly suggests targeting exogenous bacteria in LE with amoxicillin alone is not potent to result in remarkable improvement in leg stage particularly where activities engaged by most patients such as farming, charcoal burning, fishing constantly exposes them to bacterial re-infection.

The stage of the leg worsened in both patients with and without active infection who received placebo during two years of the clinical trial. There had been significant increase in the number of mossy lesions, cracks, attacks, sores, knobs and fissures on affected legs of patients in these groups. It is clear that, the already initiated complex inflammatory processes which occurred after the death of the adult worm, bacterial superinfection or probably as a result of incoming L3 larvae interacting with the host immune mechanisms

needed appropriate chemotherapeutic agent(s) to tone down the massive production of major molecular mediators known for immunological reactions thereby halting further deterioration of the leg stage (Babu *et al.,* 2009).

The few control patients who had improved leg stage is consistent with earlier report by Debrah and colleagues (2006b) where a similar result was obtained. The improvement seen can be due to the fact that these patients probably observed stringent foot care hygiene and management practices. The current data therefore suggest that in the absence of appropriate treatment for lymphedema, the pathological condition progresses quickly in majority of affected individuals. In most cases daily activities are seriously restricted as a result of increased functional impairment, persistent attacks, disability, psychological and economic burden on the affected individual and to some extent their family and the community as a whole as already reported by Gyapong and colleagues (1996) in Ghana and Ramaiah *et al* (2000) in India.

5.4 Effects of treatment on leg measurements.

To evaluate and monitor the effects of treatments on lymphedema patients, one principal technique; circumference leg measurement was used at various time points. There was no significant reduction in leg volume of most patients in all the three treatment arms.

The absence of reduction in the mean leg measurements of the doxycycline group suggest that in most lymphedema cases true reduction takes time to be observed most likely after 24 months. Mean leg measurements at 24 months were comparable with

pretreatment values. This pattern is coherent with previous report (Debrah *et al.,* 2006b) but contradicts studies by Pani and colleagues (1989) who reported greater volume reductions in patients with edema of recent onset than in those with lymphedema of longer duration. What actually accounts for non reduction in mean leg measurements in this current study is unknown. There is therefore the need to establish a more sensitive, but cost effective and simple diagnostic technique for leg measurements in LE in addition to USG technique as suggested by Dreyer *et al.,* (2002)

Within the amoxicillin group the non reduction in mean leg measurement could probable be due to the fact that the drug has no profound effects such as anti-inflammatory effects or down-regulatory mechanisms which together are believed to play essential role towards reducing leg volume. This is contrary to earlier report by (Shenoy *et al.,* 1999) where reductions of patients' leg were observed. Moreover, amoxicillin has no effects on lymph vessels functionality, as well as lymphangiogenic factors known to contribute extensively to lymphatic pathology. The drug has no known effects on the inflammatory cells believed to be predominately proliferated in the case of lymphedema and therefore does little to be desired in the reducing leg volume.

Most patients who received placebo had increase in leg measurements. However, in a few there was reduction in leg measurement during the two-year period. This pattern was actually not expected since leg volume normally increases over time without appropriate chemotherapy. What accounted for this reduction in leg measurement in these patients is not known. However, in support of earlier reports this study corroborates that circumferential measurement technique is subject to many flaws since it shows considerable variability due to transient effects such as keeping the leg elevated hours

before measurement, or the female monthly hormonal cycle (Dreyer *et al.,* 2002) therefore not reproducible.

5.5 Effects of treatment on the *Malleolus* split by CFA status

Reduction in the skin thickness at the malleolus was assessed using USG technique in all LE patients. In patients treated with 200mg/d doxycycline for six weeks, there was significant reduction at the ankle measurement in both CFA-positive and CFA-negative. From this study, it is clear that doxycycline is really important for lymphatic filariasis treatment because of its effectiveness directly or indirectly against all stages of the worm (mf, L3 and adult worm) compared to all classical anti-filarial drugs which do not achieve this feat.

Although, PCR on *Wolbachia* depletion was not done in this study, there is enough evidence in this area regarding the activities of doxycycline (Hoerauf *et al.,* 2002; 2003). Currently, several studies have distinguishingly demonstrated other possible activities of doxycycline such as reduction of collagenic activities (Sapadin *et al.,* 2006) and lymphatic vessels (Pfarr *et al.,* 2009) in addition to its profound *Wolbachia* effects which are presumably absent in patients without active infection.

Additionally, these mechanisms of action of doxycycline such as downregulating proinflammatory responses and lymphangiogenic factors (Debrah *et al*., 2006b; 2007a; Babu *et al.,* 2009) could contribute to the enhanced lymph transport in the lymph vessesl and reduced microlymphatic vessels (Fainaru *et al.,* 2008b). Moreover, doxycycline is reported to have inhibitory activities on matrix metalloprotenases which inhibit

degradative processes of collagen and elastin believed to be actively involved in tumor invasion and metastasis (Krakauer *et al.,* 2003; Sapadin *et al.,* 2006).

Interestingly, while this studies was being conducted, Soory (2008) reported reduced oxidative stress in periodontal and metabolic disease after the administration of doxycycline. Studies by Fainaru and colleagues (2008a; 2008b) have indicated that at the molecular level, doxycyclines' activity on adherence junctions in the vasculature with increased levels of VE-cadherin led to reduction in the permeability of cell between endothelial cells, thus enhancing integrity of the cell junctions. Collectively, these activities of doxycycline possibly might have resulted in reduction of the thickened skin tissue fibrosis and adipose deposition that normally characterise LE. However, further studies are needed to throw more light in this area especially on reduction of matrix metalloproteinases (MMPs) in LE.

Remarkable improvements in the CFA-negative (without active infection) lymphedema patients who apparently had no *Wolbachia* compared with CFA-positive subjects (active infection) confirms the independent function of doxycycline in lymphedema. Doxycycline is believed to have caused reduced lymph vessel density, endothelial cell proliferation and differentiation (Bennuru and Nutmann, 2009), and increased the diameter of micro-lymphatic vessels and sealed permeabilised cells, stopped further leakages of vascular endothelial growth factors and prevented relapse of pathology. These activities believed to have been controlled by doxycycline

resulted in enhanced fluid transport in the lymph vessels which led to improved condition in LE patients in addition to the drug's action on exogenous bacterial and fungal infections.

However in the CFA groupings treated both amoxicillin and placebo, there were highly significant increases in the skin thickness throughout the study time points. This result suggests that neither amoxicillin nor placebo has any effects in reducing ankle skin thickness within two years compared to doxycycline which reduces malleolus measurements in LE. These findings emphasize that doxycycline is the most appropriate drug for treating lymphedema because it could reduce ankle thickness which is very beneficial particularly in patients with early stages of LE.

5.6 Incidence of acute attacks during study period

Doxycycline had a great propensity to reduce attacks in lymphedema according to this study (Table 10) especially in early leg stage conditions. This observation is consistent with earlier reports by Debrah *et al* (2006b) where the number of attacks reduced drastically in almost fifty percent of the study patients within two years.

The reduction of acute attacks was profound in early stage lymphedema which is similar to findings by Kerketta *et al* (2005) because of few entry lesions in these individuals. However, a little over fifty percent of the patients within the doxycycline group with late LE stage continued to have acute attacks during the study period which is in agreement with a report by Suma *et al* (1997). Suma and colleagues (1997) reported that frequency of attacks is proportionally higher in the later lymphedema stage probably due to the fact

that most individuals are prone to recurrent episodes of candidasis, since their toes are closely apposed and the moisture trapped in the interdigital spaces support infection.

The achievements by doxycycline could be due to its combinatorial effects such as anti-inflammatory property (Krakauer *et al.,* 2003) and obviously antimicrobial activity (Sapadin *et al*, 2006). Antimicrobial action of doxycycline in a way reduces the presence of pathogenic bacteria which are known to cause episodic attacks. Reduction of attacks is a major relief to subjects with lymphedema because of the untold pain, discomfort and associated psychological trauma (Shenoy *et al.,* 1999) it presents. The few who continued to experience acute attacks after doxycycline treatment could be due to poor hygiene practices.

Thirty-two percent (32%) reductions of attacks in the amoxicillin group could be attributed to the drug's antimicrobial activity especially on exogenous bacterial in the streptococcus group which finds lymphedema as a suitable niche (Dreyer *et al.,* 2002; Joseph *et al.,* 2004). This finding is consistent with reports by Joseph *et al* (2004) where ADL attacks decreased significantly in oral-penicillin-treated patients but did not result in improved leg stage. Expectedly, placebo did little to prevent acute attacks in this study. Eighty-three percent of the placebo subjects experienced attacks within two years of the study. However, the few (16%) placebo patients who had no filarial attacks could be those who might have adhered to proper foot care policy (Shenoy *et al.,* 1999).

Furthermore, proper foot care practice such as feet washing and daily exercise of the affected limb drastically reduce attacks normally associate with LE and therefore should be encouraged especially in the absence of therapy (Shenoy *et al.,* 1999) although this contradicts report by Taylor *et al* (1997) which suggest that in many areas of endemicity

where local hygiene is not maintained, there is no concurrent increase in pathology. In comparing all the treatment arms, doxycycline had a distinguishing ability to decrease attacks in lymphedema patients followed by amoxicillin.

5.7 Assessment of lymphedema staging-improvement halts of progression, pathology progression at 24 months after treatment

Considering improvement in lymphedema stage, halt of pathology progression and progression within 24 months after treatment among the three treatment arms, doxycycline was the best followed by amoxicillin and finally placebo. In majority of the patients who received six weeks doxycycline treatment, there was a highly significant improvement (43.9%) in the leg stage at the end of the study (Table 10).

A significant number of the LE patients in the doxycycline group had their pathology halted (43.9%) (p=0.006, Pearson Chi Square). The effects of doxycycline can possibly be attributed to several factors like the already established anti-VEGF, antimicrobial and anti-inflammatory activities. Possible reduction of cytokines and proinflammatory mediators as suggested by several studies by Debrah *et al* (2006b; 2007a; 2009) might play essential role in observed improved pathology.

Again experimental studies in mice by Fainaru *et al* (2008a; 2008b) have shown that doxycycline result in reduced micro-vascular density and sealed lymph vessels. These effects of doxycycline could contribute to efficient fluid transport processes in lymphedema leading to improved leg stage and halt of pathology in the doxycycline-treated group. Therefore in this current study it is believed that doxycycline caused enviable improvement in leg stage, halted and prevented progression of leg stage in most LE within 24 months post treatment.

Regarding progression of leg stage, only 5 out of 41 in the doxycycline group had their pathological conditions progressed. The five who had progression of pathology had late leg stage (5) even before treatment and most of the time it is difficult to see improvement in the later stages of LE. Moreover re-infection and poor hygiene practices in these subjects could account for progressed pathology in the few doxycycline-treated patients.

According to this study, amoxicillin resulted in excellent halt of progression of disease in most patients (Table 10). The drug is known to disrupt bacterial cell wall especially the gram positives such as the streptococcus group reducing their possible ability to worsen disease state (Friedland and McCracken, 1994). At the end of the study, only 1 person out of 31treated with amoxicillin had improved leg condition whereas 19 out of 31 halted disease progression. Eleven out of 31 representing (35.4%) who received amoxicillin had increase in progression of leg stage within two years of the study in contrary to the earlier report by Shenoy *et al* (1999).

In a similar investigation penicillin plus foot care practice resulted in improved condition in brugian LE compared to DEC plus penicillin (Shenoy *et al.,* 1999). It is possible that the extent of pathological conditions, study design used and even doses of penicillin administered might account for the differences seen in comparison to this study. The high proportion in terms of halt of disease progression achieved by amoxicillin is perhaps due to its antimicrobial activity as reported (Friedland and McCracken, 1994)

Obviously, the placebo group recorded the highest number of patients with progressed pathological conditions (Table 10). Although 12 patients who received placebo had halt of progression, 2 patients had improved condition; this is in line with previous studies

(Shenoy *et al.,* 1999; Debrah *et al.,* 2006b). It is possible the 12 patients who had halt of disease progression and the 2 who had improved pathology observed daily foot care hygiene practices. These findings clearly suggest the need to treat lymphedema subjects with an appropriate anti-LE therapy since the pathology deteriorates with time in absence of a therapy.

CHAPTER 6

6.0 CONCLUSIONS

The study was carried out purposefully to define the role of doxycycline treatment in filarial lymphedema subjects with and without active infection in comparison with amoxicillin. Interestingly, the present data showed a highly significant amelioration in leg condition between doxycycline and amoxicillin/placebo but not between amoxicillin versus placebo.

Treatment with 200mg per day of doxycycline for six weeks caused a highly significant reversal and/or prevented progression of lymphedema particularly in the early stage at the foot level of those without active infection. Doxycycline treatment, additionally, resulted in reduced mean ankle skin measurement, reduced acute filarial attack and finally caused improved condition in most of the LE patients compared with amoxicillin and placebo. Therefore, doxycycline unequivocally proves to be the first chemotherapy for treating filarial pathology particularly lymphedema because it has an unprecedented ability to complete sterilizing effects and reduced antigenaemia levels compared with amoxicillin and placebo groups.

Again the combinatorial and multiple effective targets of doxycycline (such as activity on *Wolbachia*, exogenous bacterial, VEGFs, anti-inflammatory and antimicrobial) make it an appropriate anti-LE therapy than current antifilarial drugs. These multiple targets give doxycycline a competitive edge compared with amoxicillin which targets only exogenous bacterial.

Contrarily, there was minimal improvement in LE patients treated with doxycycline who had active infection compared to those without infection according to the data. There is the need for further studies to be conducted regarding the role of doxycycline in subjects with active infection. From the current study it is obvious that doxycycline has other targets apart from its known effects on the *Wolbachia* endosymbionts.

Although the current study shows that 1000mg of amoxicillin per day for 6 weeks halt disease progression with considerable reduction of acute filarial attacks normally associated with lymphedema, doxycycline has a higher proportional effect in reduction of acute attacks according to the data from this study. Additionally, doxycycline targets both *Wolbachia* and exogenous bacterial whereas amoxicillin targets only exogenous bacterial. Therefore, it is worth treating lymphedema with doxycycline than with amoxicillin because currently it is the only drug with considerable ameliorative effects against filarial LE and non-filarial LE (podonocosis). It is also currently, the only potentially useful drugs for other clinical state (those active and without active infection) in lymphatic filariasis. Nevertheless, given that amoxicillin considerably results in halt of disease progression in LE subjects, the compound should be studied in combination with other drugs and/or with other doses.

Regarding the excruciating pains individuals with lymphedema go through, it is expected that a six-week course of 200mg/d of doxycycline will be better appreciated. Doxycycline-treated LE patients who had improved conditions returned to their former work such as fishing, farming and other daily activities which were approached with difficulty before the onset of the treatment.

Doxycycline treatment has proven to be a better option for treating LE since current filarial drugs do little for individuals with lymphedema and also because doxycycline is an already licensed

drug used in endemic regions. Although several independent scientific findings support the anti-inflammatory, anti-microbial, matrix metalloproteinase and immunomodulatory effects of doxycycline, further investigations must be carried out, at both the laboratory and clinical levels to corroborate these properties in filarial lymphedema.

6.1 RECOMMENDATIONS

It is recommend that:

- 200mg of doxycycline per day for 6 weeks should be administered to individuals with lymphedema in endemic regions since it can be used for almost all the clinical groups in filariasis
- More investigations are needed to establish the activity of doxycycline at the molecular level in lymphedema particularly in the area of VEGF since *Wolbachia* per se might not be the primary target in LE considering the fact that about 60% of the subjects are without active infection.
- Repeated treatments of lymphedema patients with 200mg/d should be considered for future research.
- Effects of other drugs such as rifampicin (since it can be administered to children) should be assessed in LE addition to doxycycline thus bridging one of the contraindications of doxycycline.
- Proper foot care policies should be adhered to especially in the absence of a recommended therapy.
- Education should be given to all residents in endemic areas to report early stages of LE.

REFERENCES

1. **Achen M, Jeltsch M, Kukk E, Makinen T, Vitali A, Wilks A, Alitalo K and Stacker S (1998)** Vascular endothelial growth factor D (VEGF-D) is a ligand for the tyrosine kinases VEGF receptor 2 (Flk1) and VEGF receptor 3 (Flt4). *Proc Natl Acad Sci USA* **95**: 548-553.

2. **Addiss DG, Eberhard MI, Lammie, PJ (1994)** 'Filarial' adenolymphangitis without filarial infection. *Lancet* **343** (8897):597.

3. **Ahorlu CK, Dunyo SK, Koram KA, Nkrumah FK, Aagaard-Hansen J, Simonsen PE (1999)** Lymphatic filariasis related perceptions and practices on the coast of Ghana. Implication for prevention and control. *Acta Trop* **73**:251-64.

4. **Ahorlu CK, Dunyo SK, Asamoah G and Simonsen PE (2001)** Consequences of hydrocele and the benefits of hydroceloctomy: a qualitative study in lymphatic filariasis endemic community on the Coast of Ghana. *Acta. Trop*. **80**:215-221

5. **Amaral F, Dreyer G, Figueredo-Silva J, *et al*. (1994)** Live adult worms detected by ultrasonography in human bancroftian filariasis. *Am J Trop Med Hyg* **50**: 753-7.

6. **Awadzi K, Addy ET, Opoku NO, Plenge-Bönig A, Büttner DW (1995)** The chemotherapy of onchocerciasis XX: ivermectin in combination with albendazole. *Trop Med Parasitol* **46**:213-20.

7. **Aziz MA, Diallo S, Diop IM, Lariviere M, and Porta M (1982)** Efficacy and tolerance of ivermectin in human Onchocerciasis. *Lancet*, ii: **171**-173.

8. **Babu S, Bhat SQ, Pavan Kumar N, Lipira AB, Kuma S, *et al,* (2009)** Filarial Lymphedema is characterized by Antigen-Specific Th1 and Th17 Proinflammatory Responses and a Lack of Regulatory T Cells. *PLoS Negl Trop Dis* **3** (4)

9. **Behbehani K (1998)** Candidate parasitic diseases. *Bull Wld Hlth Org*. **76** (Suppl 2):64-67.

10. **Bennuru S and Nutman TB (2009)** Lymphangiogenesis and Lymphatic Remodeling Induced by Filarial Parasites: Implications for Pathogenesis. *PLoS Pathog* **5**(12).

11. **Binoy C, Rao VG, Ananthakriashnan N, Kate V, Yuvraji J and Pani SP (1998)** Oentoplasty in the mangement of filarial lymphedema. *Trans of R Socy of Trol Med Hyg* **92**, 317-319.

12. **Bonnetblanc J and Bedane C (2003)** Erysipelas- recognition and management. *Am J Clin Dermatol.* **4**:157-163.

13. **Brattig NW, Rathjens U, Ernst M, Geisinger F, Tischendorf, FW (2000)** Lipopolysaccharide-like molecules derived from *Wolbachia* endobacteria of the filaria *Onchocerca volvulus* are candidate mediators in the sequence of inflammatory and anti-inflammatory responses of human monocytes. *Microbes Infect* **2**:1147-57.

14. **Brattig NW, Büttner DW, Hoerauf A (2001)** Neutrophil accumulation around *Onchocerca* worms and chemotaxis of neutrophils are dependent on *Wolbachia* endobacteria. *Microbes Infect* **3**:439-46.

15. **Brattig NW, Bazzocchi C, Kirschning CJ, Reiling N, Buttner DW, *et al*. (2004)** The major surface protein of Wolbachia endosymbionts in filarial nematodeselicits immune responses through TLR2 and TLR4. *J Immunol* **173**: 437-445

16. **Burkhart CN and Burkhart CG (1999)** Ivermectin: a few caveats are warranted before initiating therapy for scabies. *Arch Dermatol* **135**:1549-50.

17. **Burton GJ (1963)** Natural and experimental infection of bedbugs with *Wuchereria bancrofti* in British Guiana. *Am J Trop Med Hyg* **12**:541-547.

18. **Campbell W (1991)**. Ivermectin as an anti-parasite agent for use in humans. *Annu. Rev. Microbiol.* **45**, 445–74.

19. **Casley-Smith JR, Jamal S and Casley-Smith RJ (1993)** Reduction of filaritic lymphedema and elephantiasis by 5, 6-benzoalphapyne (couramin), and the effects of DEC. *Ann Trop Med and Parasitol* **87**, 247-258.

20. **Carme B, Utahia A, Tuira E and Teuru T (1979)** Filarial elephantiasis in French Polynesia: a study concerning the beliefs of 127 patients about the origins of their disease. *Trans R Soc Trop Med Hyg* **73**:424-426.

21. **Cox FEG (2000)** Elimination of lymphatic filariasis as a public health problem. *Parasitol Today* **4**: 135-137.

22. **Das LK, Reddy GS, Pani SP (2003)** Some observations on the effect of Daflon (micronized purified flavonoid fraction of Rutaceae aurantiae) in Bancroftian filarial lymphedema. *Filaria J*. **2**, 5.

23. **Dasgupta A (1984)** Cursory overview of lymphatic filariasis. *Ind J Pathol & Micro* **27**:273-280.

24. **Debrah AY, Mand S, Marfo-Debrekyei Y, Larbi J, Adjei O and Hoerauf A (2006a)** Assessment of microfilarial loads in the skin of onchocerciasis patients after treatment with different regimens of doxycycline plus ivermectin. *Filaria Journal* **5**, 1.

25. **Debrah AY, Mand S, Specht S, *et al.* (2006b)** Doxycycline reduces plasma VEGF-C / sVEGFR-3 and improves pathology in lymphatic filariasis. *PLoS Pathogens* **2**, 92.

26. **Debrah AY, Mand S, *et al.* (2007a)** "Plasma vascular endothelial growth Factor-A (VEGF-A) and VEGF-A gene polymorphism are associated with hydrocele development in lymphatic filariasis." *Am J Trop Med Hyg* **77**(4): 601-8.

27. **Debrah AY, Mand S, *et al.* (2007b)** "Macrofilaricidal effect of 4 weeks of treatment with doxycycline on Wuchereria bancrofti." *Trop Med Int Health* **12**(12): 1433-41.

28. **Debrah AY**, **Mand S**, **Marfo-Debrekyei Y**, **Batsa L**, **Pfarr KM**, **Lawson B, Taylor MJ**, **Adjei O**, **Hoerauf A (2009)** Reduction in levels of plasma vascular endothelial growth factor-A and improvement in hydrocele patients by targeting endosymbiotic *Wolbachia* sp. in *Wuchereria bancrofti* with doxycycline. *Am J Trop Med. Hyg* **80** (6):956-63.

29. **Dreyer G, Addiss D, Noroes J, Amaral F, Rocha A, Coutinho A (1996)** Ultrasonic assessment of the adulticidal efficacy of repeat high-dose ivermectin in Bancroftian filariasis. *Trop Med and Int Hlth* **1**:427-432.

30. **Dreyer, G, Noroes, J, Addiss, D (1997)** The silent burden of sexual disability associated with lymphatic filariasis. *Acta Tropica* **63**:57-60

31. **Dreyer G, Medeiros Z, Netto MJ, Leal NC, de Castro LG, Piessens WF (1999)** Acute attacks in the extremities of persons living in an area endemic for bancroftian filariasis: Differentiation of two syndromes. *Trans R Soc Trop Med Hyg.* **93**:413-417.

32. **Dreyer G, Noroes J, Figueredo-Silva J, Piessens WF (2000a)** Pathogenesis of lymphatic disease in bancroftian filariasis: a clinical perspective. *Parasitol Today* **16**:544-8.

33. **Dreyer G, Addiss D, Dreyer P, Noroes J (2002b)** *Basic Lymphedema Management:treatment and prevention of problems associated with lymphatic filariasis* Hollis, NH: Hollis Publishing Co. page 112.

34. **Dreyer G and Piessens WF (2002)** In: *Lymphatic filariasis.* Tropical Medicine Science and Practice, Vol. 1. (Ed. T.B. Nutman; Series eds. Pasvol, G. & Hoffman, S.L.), pp 245-257. London: Imperial College Press.

35. **Dunyo SK, Appawu M, Nkrumah FK, Baffoe-Wilmot A, Perdeson EM and Simonsen PE (1996)** Lymphatic filariasis in coastal Ghana. *Trans R Soc Trop Med Hyg*, **90:** 634-638.

36. **Dunyo S, Nkrumah F, Simonsen P (2000)** Single-dose treatment of Wuchereria bancrofti infections with ivermectin and albendazole alone or in combination:

evaluation of the potential for control at 12 months after treatment. *Trans. R. Soc. Trop. Med. Hyg*. **94**, 437-43.

37. **Evans DB, Gelband H, Vlassoff C (1993)** Social and economic factors and the control of lymphatic filariasis: a review. *Acta Trop.* **53**(1):1-26.

38. **Eberhard M, Hightower A, Addiss, D, Lammie M (1997)** Clearance of Wuchereria bancrofti antigen after treatment with diethylcarbamazine or ivermectin. *Am J Trop Med Hyg* **57**, 483-6.

39. **Fainaru O, Adini I, Benny O, *et al.* (2008a)** Doxycycline induces membrane expression of VE-cadherin on endothelial cells and prevents vascular hyperpermeability. *FASEB* **22**: 3728-3735.

40. **Fainaru O, Hornstein, MD, *et al.* (2008b)** "Doxycycline inhibits vascular leakage and prevents ovarian hyperstimulation syndrome in a murine model." *Fertil Steril*. **92** 1701-1705

41. **Ferrara N (2000)** VEGF: an update on biological and therapeutic aspects. *Curr Opin Biotechnol* 11: 617-624.

42. **Figueredo-Silva J, Jungman P, Noroes J, Piessens W, Coutinho A, Brito C, Rocha A, and Dreyer G (1996)** Histological evidence for adulticidal effect of low doses of diethylcarbamazine in Bancroftian filariasis. *Trans R Soc Trop Med Hyg* **90**, 192-4.

43. **Figueredo-Silva J, Noroes J, Cedenho A and Dreyer G (2002)** The histopathology of bancroftian filariasis revisited: the role of the adult worm in the lymphatic-vessel disease. *Ann Trop Med Parasitol* **96***:* 531-541.

44. **Francis H, Awadzi K and Ottesen EA (1985)** The Mazotti reaction following treatment of onchocerciasis with diethylcarbamazine: clinical severity as a function of infection intensity. *Am J Trop Med Hyg* **34**:529-536.

45. **Freedman DO, Bui T, De Almeida Filho PJ, Braga C, Maiae Silva MC, Maciel A and Furtado AF (1995)** Lymphoscintigraphic assessment of the effect of diethylcarbamazine treatment on lymphatic damage in human Bancroftian filariasis. *Am J Trop Med Hyg* **52**, 258-261.

46. **Friedland IR and McCracken GH, Jr (1994)** Management of infections caused by antibiotic-resistant *Streptococcus pneumoniae. New Engl J Med.,* **331***:377*-382.

47. **Goa KL, McTavish D and Clissold SP (1991)** Ivermectin. A review of its antifilarial activity, pharmacokinetic properties and clinical efficiency in Onchocerciasis. *Drugs* 42:640-658.

48. **Goe FB, Janet SB and Stephen AM (2004)** *Medical Microbiology*, Twenty Third Edition. Pages 163,184 and185

49. **Gyapong JO, Gyapong M, Evans DB, Aikins MK and Adjei S (1996)** The economic burden of lymphatic filariasis in northern Ghana. *Ann Trop Med Parasitol,* **90**:39-48.

50. **Gyapong JO, Webber RH, Morris J and Bennett S (1998)** Prevalence of hydrocele as a rapid diagnostic index for lymphatic filariasis. *Trans R Soc Trop Med Hyg.* **92**:40-43.

51. **Hawking F (1962)** Microfilaria infestation as an instance of periodic phenomenon seen host parasite relationships. *Ann New York Acad Sci* **98**:940-953.

52. **Hoerauf A, Nissen-Pahle K, Schmetz C, Henkle-Duhrsen K, Blaxter ML, Buttner DW, *et al.* (1999)** Tetracycline therapy targets intracellular bacteria in the filarial nematode *Litomosoides sigmodontis* and results in filarial infertility. *J Clin Invest* **103:** 11-18.

53. **Hoerauf A, Volkmann L, Hamelmann C, Adjei O, Autenrieth IB, Fliescher B, *et al*. (2000)** Endosymbiotic bacteria in worms as targets for a novel chemotherapy in filariasis. *Lancet 355*: 1242-3.

54. **Hoerauf A, Mand S, Adjei O, Fleischer B and Büttner DW (2001)** Depletion of *Wolbachia* endobacteria in *Onchocerca volvulus* by doxycycline and microfiladermia after ivermectin treatment. *Lancet* **357** : 1415-6.

55. **Hoerauf A, Adjei O and Buttner DW (2002)** Antibiotics for the treatment of onchocerciasis and other filarial infections. *Curr Opin Investig Drugs* **3**:533-537.

56. **Hoerauf A, Mand S, Volkmann L, Büttner M, Marfo- Debrekyei Y, Taylor M, *et al*. (2003)** Doxycycline in the treatment of human onchocerciasis: Kinetics of *Wolbachia* endobacteria reduction and of inhibition of embryogenesis in female *Onchocerca* worms. *Microbes Infect* **5***:*261-73.

57. **Hoerauf A, Specht S, Büttner M, Pfarr K, Mand S, Fimmers R, Marfo Debrekyei Y, Konadu P, Debrah AY, Bandi C, Brattig N, Albers A, Larbi J, Batsa L, Adjei O and Büttner DW (2008)** *Wolbachia* endobacteria depletion by doxycycline as antifilarial therapy has macrofilaricidal activity in onchocerciasis: a randomized placebo-controlled study. *Med Microbiol Immunol* **197**:295-31.

58. **Horton J (2000)** Albendazole: a review of antihelminthic efficacy and safety in humans. *Parasitology* **121**(Suppl.):S113-S132.

59. **Horton J, Witt C, Ottesen EA *et al.* (2002)** An analysis of the safety of the single dose, two drug regimens used in programmes to eliminate lymphatic filariasis. *Parasitol.* **121**(Suppl.):S147-S160.

60. **Ismail MM, Jayakody RL, Weil GJ, *et al.* (1998)** Efficacy of single dose combinations of albendazole, ivermectin and diethylcarbamazine for the treatment of bancroftian filariasis. *Trans R Soc Trop Med Hyg* **92:** 94-97.

61. **Jeltsch M, Kaipainen A, Joukov V, Meng X, Lakso M, Rauvala H, Swartz M, Fukumura D, Jain RK and Alitalo K (1997)** Hyperplasia of lymphatic vessels in VEGF-C transgenic mice. *Science* **276**: 1423-1425.

62. **Joseph A, Mony PM, Prasad M, John S, Srikanth and Mathai D (2004)** The efficacies of affected-limb care with penicillin, diethylcarbamazine, the combination of both drugs or antibiotic ointment, in the prevention of acute adenolymphangitis during bancroftian filariasis *Ann Trop Med Parasitol*, **98,** 7, 685-696.

63. **Kaipainen A, Korhonen J, Mustonen T, Van Hinsbergh VWM, Fang GH, Dumont D, Breitman M and Alitalo K (1995)** Expression of the *Fms*-Like Tyrosine kinase FLT4 gene becomes restricted to lymphatic endothelium during development. *Proc Natl Acad Sci USA* **92**: 3566-3570.

64. **Karkkainen MJ, Makinen T and Alitalo K (2002)** Lymphatic endothelium: a new frontier of metastasis research. *Nat Cell Biol* **4***:* E2-E5.

65. **Kerketta AS, Babu BV, Rath K, Jangid PK, Nayak AN, and Kar SK (2005)** A randomized clinical trial to compare the efficacy of three treatment regimens along with foot care in the morbidity management of filarial lymphoedema. *Trop Med Int'l Health* vol **10** no **7** pp 698–705

66. **Korpelainen EI and AlitaloK (1998)** Signaling angiogenesis and lymphangiogenesis. *Curr Opin Cell Biol* **10**: 159-164.

67. **Krakauer T and Buckley M (2003**) Doxycycline Is Anti-Inflammatory and Inhibits Staphylococcal Exotoxin-Induced Cytokines and Chemokines. *Antimicro and chemo,* p.3630-3633

68. **Kukk E, Lymboussaki A, Taira S, Kaipainen A, Jeltsch M, Joukov V and Alitalo K (1996)** VEGF-C receptor binding and pattern of expression with VEGFR-3 suggests a role in lymphatic vascular development. *Development* **122:** 3829-3837.

69. **Kumaraswami V (2000)** The clinical manifestations of lymphatic filariasis. *Tropical medicine in science and practice*. Volume 1. London: Imperial College Press. pp. 103-12.

70. **Lacey E, Brady RL, Pritchard RK, Watson TR (1987)** Comparision of inhibition of polymerase of mammalian tubulin: helminth ovicidal activity by benzimidazole carbamates. *Vert. Parasitol* **23**, 105-119.

71. **Makinen T, Veikkola T, Mustjoki S, Karpanen T, Catimel B, Nice EC, Wise L, Mercer A, Kowalski H, Kerjaschki D, Stacker SA, Achen MG, and Alitalo K (2001)** Isolated lymphatic endothelial cells transduce growth, survival and migratory signals via the VEGF-C/D receptor VEGFR-3. *EMBO J* **20**: 4762-4773.

72. **Maizels RM and Denham DA (1992**) Diethylcarbamazine (DEC): immuno pharmacological interactions if an antifilarial drug. *Parasitol;* **105** (suppl):S49-S60.

73. **Mand S, Marfo-Debrekyei Y, Dittrich M, Fischer K, Adjei O,Hoerauf A, (2003).** Animated documentation of the filaria dance sign (FDS) in bancroftian filariasis. *Filaria J 2:* 3.

74. **McGuinness CL and Burnand KG (2001)** Lymphedema. *Trop. Doct.* **31**, 2-7.

75. **Michael E, Bundy DA and Grenfell BT (1996)** Re-assessing the global prevalence and distribution of lymphatic filariasis. *Parasitol.***112**:409-428.

76. **Michael E and Bundy DAP (1997)** Global mapping of lymphatic filariasis**.** *Parasitol. Today* **13** 472-476.

77. **Molyneux DH and Taylor MJ (2001)** Current status and future prospects of the global lymphatic filariasis programme.*Curr Opin Infect Dis.* **14**:155-15.

78. **Nanduri J and Kazura JW (1989)** Clinical and laboratory aspects of filariasis. *Clinl Microbiol* **2**:39-50.

79. **Olszewski W, Jamal S, Manokaran G, Lukomska B, Kubicka U (1993)** Skin changes in filarial and non-filarial lymphedema of the lower extremities. *Trop Med Parasitol* **44**:40-41.

80. **Olszewski WL and Jamal S (1994)** Skin bacterial factor in progression of filarial lymphedema. *Lymphology.* **27**:148-149.

81. **Olszewski WL, Jamal S, Manokaran G, Pani S, Kumaraswami V, Kubica U, Lukomska B, Dworczynski A, Swoboda E, Meisel-Mikolajczyk E (1997)** Bacteriologic studies of skin, tissue fluid, lymph and lymph nodes in patients with filarial lymphedema. *Am J Trop Med Hyg* **57**: 7-15.

82. **Olszewski WL, Jamal S, Manokaran G, Pani S, Kumaraswami V, Kubicka U, Lukomska B, Tripathi FM, Swoboda E, Meisel-Mikolajczyk F, Stelmach E, Zaleska M (1999)** Bacteriological studies of blood, tissue fluid, lymph and lymph nodes in patients with acute dermatolymphangioadenitis (DLA) in course of filarial lymphedema. *Acta Trop.***73**:217-224.

83. **Ottesen EA (1980)** Immunopathology of Lymphatic Filariasis in Man. *Springer Semin. Immunopathol.* **2**.373-385.

84. **Ottesen EA (1984**) Immunological aspects of lymphatic filariasis and onchcerciasis in man. *Trans R Soc Trop Med Hyg* **78** (supplement):9-18.

85. **Ottesen EA (1985)** Efficacy of diethylcarbamizine in eradicating infection with lymphatic-dwelling filariae in humans. *Rev Infect Dis* **7**:341-356.

86. **Ottesen EA (1989)** Filariasis now. *Am J Trop Med Hyg* **41** *(supplement)*: **9**-17.

87. **Ottesen EA (1993)** Filarial infections. *Infec Dis Clin North Amer* **7**:619-633.

88. **Ottesen EA (1995)** Immune responsiveness and the pathogenesis of human onchocerciasis. *J Infec Dis* **171**:659-671.

89. **Ottesen EA, Ramachandran C (1995)** Lymphatic filariasis infection and disease: control strategies. *Parasitol. Today* **11**, 129-31.

90. **Ottesen EA, Duke BO, Karam M and Behbehani K (1997)** Strategies and tools for the control/elimination of lymphatic filariasis. *Bull Wld Hlth Org*. **75**:491-503.

91. **Ottesen EA, Ismail MM, Horton J (1999)** The role of albendazole in programmes to eliminate lymphatic filariasis. *Parasitol. Today* **15**, 382-386.

92. **Ottesen EA (2000)** The global programme to eliminate lymphatic filariasis**.** *Trop Med Int Health* **5**:591-4.

93. **Oxberry ME, Geary TG, Winterrowd CA, Pritchard RK (2001)** Individual expression of recombinant and tubulin from *Haemonchus contortus*: polymerization and drug effects. *Protein Expr*. Purify. **21**, 30-39.

94. **Pani SP, Krishnamoorthy K, Prathibha J, Rao AS (1989)** Diethylcarbamazine and supportive measures for the treatment of brugian filariasis**.** *Natl Med J India* 89**(2):**260-263.

95. **Pani S, Yuvaraj J, Vanamail P, Dhanda V, Michael E, Grenfell BT, Bundy DAP (1995)** Episodic adenolymphangitis and lymphoedema in patients with bancroftian filariasis. *Trans R Soc Trop Med Hyg*. **89**:72-74.

96. **Pani S, Subramanyam, Reddy G, Das PK, Vanamail P, Hoti S, Ramesh J, Das P (2002)** Tolerability and efficacy of single dose albendazole, diethylcarbamazine citrate (DEC) or coadministration of albendazole with DEC in the clearance of *Wuchereria bancrofti* in asymptomatic microfilaraemic volunteers in Pondicherry, South India: a hospital-based study. *Filaria J* **1***:* 1.

97. **Partono F (1987).** The spectrum of disease in lymphatic filariasis. *Filariasis*: Ciba Foundation Symposium , 127:15-31.

98. **Pfarr KM, Debrah AY, Specth S, Hoerauf A (2009)** Filariasis and lymphedema *Parasit Immunol*, **31**.1-9

99. **Plaisier AP, Stolk WA, van Oortmarssen GJ, Habbema JD (2000**) Effectiveness of annual ivermectin treatment for Wuchereria bancrofti infection. *Parasitol Today* **16**:298-302.

100. **Punkosdy GA, Addiss DG and Lammie PJ (2003)** Characterization of antibody responses to *Wolbachia* surface protein in humans with lymphatic filariasis. *Infect Immu*, **71**, 5104-5114.

101. **Rajan TV (1998**) A hypothesis for the tissue specificity of nematode parasites. *Exp Parasitol* **89**: 140–142.

102. **Ramaiah K D, Radhamani MP, John KR, Evans DB, Guyatt H, Joseph A, Datta M and Vanamail P (2000)** The impact of lymphatic filariasis on labour inputs in southern India: results of a multi-site study. *Ann Trop Med Parasit*, **94**, 353–364.

103. **Roberts LS and Janovy J (1997)** Nematodes: Filaroidea, the filarial worms In: Schmidt GD, Robers LS. *Foundations of Parasitology* 5th. Edition, chapter 29.WCB publishers Boston.

104. **Rockson SG (2001**) Lymphedema. *Am J Med* 288-295.

105. **Sapadin AN and Fleischmajer R (2006)** Tetracyclines: Nonantibiotic properties and their implications. *J Am Acad Dermatol* **54**: 258-65.

106. **Shenoy RK, Suma TK, Rajan K, Kumaraswami V (1998)** Prevention of acute adenolymphangitis in brugian filariasis: comparison of the efficacy of ivermectin and diethylcarbamazine each combined with local treatment of the affected limb. Ann *Trop Med Parasitol* **92**: 587-594.

107. **Shenoy RK, Kumaraswami V, Suma TK, *et al.* (1999)** A double blind placebo controlled study of the efficacy of oral penicillin, diethylcarbamazine or local treatment of the affected limb in preventing acute adenolymphangitis in lymphoedema caused by brugian filariasis. *Ann Trop Med Parasitol* **93**: 367-377.

108. **Smith HL and Rajan TV (2000)** Tetracycline inhibits development of the infective-stage larvae of filarial nematodes *in vitro. Exp Parasitol* **95:**265-70.

109. **Soory M (2008)** A Role for Non-Antimicrobial Actions of Tetracyclines in Combating Oxidative Stress in Periodontal and Metabolic Diseases **2***,* 5-12.

110. **Stephanie AR, Els Mathieu, Addiss DG, Sodahlon YK (2007)** A survey of treatment practices and burden of lymphedema in Togo. *Trans R Soc Trop Med Hyg***101**, 391-397.

111. **Tabibiazar R, Cheung L, Han J, Swanson J, Beilhack A, *et al.* (2006)** Inflammatory manifestations of experimental lymphaticinsufficiency. *PLoS Med* 3(**7**).

112. **Taylor MJ and Turner PF (1997)** Control of lymphatic filariasis. *Parasitol. Today* **13**:85 87.

113. **Taylor MJ, Cross HF, Bilo K (2000)** Inflammatory responses induced by the filarial nematode *Brugia malayi* are mediated by lipopolysaccharide-like activity from endosymbiotic *Wolbachia* bacteria. *J Exp Med* **191**:1429-36.

114. **Taylor MJ, Cross HF, Ford L, Makunde WH, Prasad GB, *et al.* (2001)** *Wolbachia* bacteria in filarial immunity and disease. *Parasite Immunol* **23**:401-409.

115. **Taylor MJ, Makunde WH, McGarry HF, Turner JD, Mand S and Hoerauf A (2005)** Macrofilaricidal activity after doxycycline treatment of *Wuchereria bancrofti*: a double-blind, randomised placebo-controlled trial. *Lancet* **365**: 2116-2121.

116. **Townson S, Hutton D, Siemienska J, Hollick L, Scanlon T, Tagboto SK and Taylor MJ (2000)** Antibiotics and *Wolbachia* in filarial nematodes: antifilarial activity of rifampicin, oxytetracycline and chloramphenicol against *Onchocerca gutturosa*, *Onchocerca lienalis* and *Brugia pahangi*. *Ann Trop Med Parasitol* **94:** 801-816.

117. **Turner JD, Tendongfor N, Esum M, Johnston KL, Langley RS, *et al.* (2010)** Macrofilaricidal Activity after Doxycycline Only Treatment of Onchocerca volvulus in an Area of Loa loa Co-Endemicity: A Randomized Controlled Trial. *PLoS Negl Trop Dis* 4(**4**): e660.

118. **Veikkola T, Jussila L, Makinen T, Karpanen T, Jeltsch M, Petrova TV, Kubo H, Thurston G, Mcdonald DM, Achen MG, Stacker SA, and Alitalo K (2001)** Signalling via vascular endothelial growth factor receptor-3 is sufficient for lymphangiogenesis in transgenic mice. *EMBO J* **6**: 1223-1231.

119. **Whithworth JA, Morgan D, Maude GH, Downham MD, Taylor DW (1991)** A community trial of ivermectin for onchocerciasis in Sierra Leone: clinical and parasitological responses to the initial dose. *Trans R Soc Trop Med Hyg* **85**:92-96.

120. **WHA (1997)** Elimination of Lymphatic Filariasis as a public health *WHA, Geneva A50.**29.***

121. **WHO (1996)** Coumarin (Lodema). WHO Pharmaceutical Newsletter **10**, 2.

122. **WHO (1999)** Building partnerships for lymphatic filariasis. Strategic plan. Geneva: *WHO/FIL*/**99**.198.

123. **WHO (2002)** Global programme to eliminate lymphatic filariasis. CDS/CPE/CEE/**33.**

124. **WHO (2006)** Informal consultation on preventing disability from lymphatic filariasis, *Wkly Epidemiol Rec.*; **81**:373–384.

125. **WHO (2009)** Global programme to eliminate lymphatic filariasis**.** *Wkly. Epidemiol. Rec* **84**(42), 437-444.

126. www.parasiticdisesases.org, visited on 06/07/2009; 17:03.

127. www.wilkapedia.org

128. www.encarta.com

129. www.cdc.org

130. **Zelikovski A, Deutsch A, Reiss R (1993)** The sequential pneumatic compression device in surgery for lymphedema in the limbs. *J Cardio Surg (Tarino)* **24,** 122–126.