KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY, KUMASI COLLEGE OF HEALTH SCIENCES SCHOOL OF MEDICAL SCIENCES DEPARTMENT OF CLINICAL MICROBIOLOGY

MICROFILARIDERMIA ASSESSMENT OF THE EFFICACY OF IVERMECTIN ALONE AND IVERMECTIN PLUS ALBENDAZOLE AGAINST ONCHOCERCIASIS.

By

YUSIF MUBARIK

AUGUST, 2015

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A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF PHILOSOPHY

In the Department of Clinical Microbiology, School of Medical Sciences, College of Health Sciences

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DECLARATION

I declare that this thesis is my own work towards the award of an MPhil in Clinical Microbiology. It does not contain any materials previously published by another person. This work has not been submitted for the award of any other degree in any university, except where due acknowledgement has been made in the text.

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(STUDENT)

……. ……..

…… SIGNATURE Date

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PROF. E.H. FRIMPONG Date

(HEAD OF DEPARTMENT)

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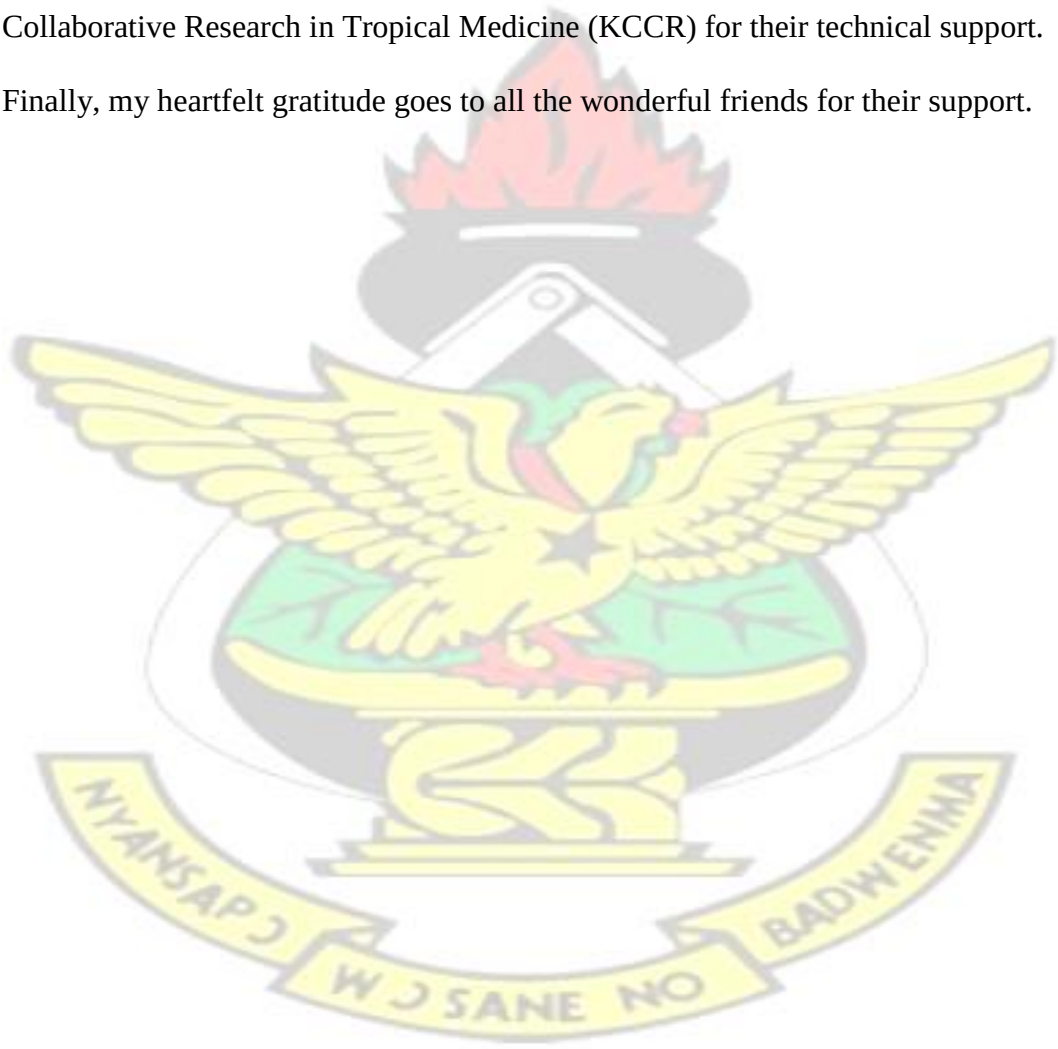

DEDICATION

I dedicate this work to

God, my family and

friends.

ABSTRACT

Onchocerciasis, commonly known as river blindness, is a vector-borne parasitic disease which affects approximately 37 million people world wide, mostly in subSaharan African countries. In Ghana, the standard treatment of onchocerciasis is annual mass drug administration with ivermectin at a dose of 150-200µg/kg. However, this regimen kills only microfilariae and therefore repopulation of microfilariae by adult female worms resumes 3 to 6 months after ivermectin treatment. In this study, an open-labelled clinical trial was conducted using microfilariae levels to assess the efficacy of ivermectin alone and ivermectin plus albendazole given annually and semi-annually. A total of 272 onchocerciasisinfected volunteers were randomised into ivermectin alone annually, ivermectin alone semi-annually, ivermectin plus albendazole annually and ivermectin plus albendazole semi-annual treatment arms. Participants in the annual treatment arms received vitamin C at 6 months. Microfilariae loads of all study volunteers were monitored at pre-treatment, 6 months and 18 months using skin biopsies. All four treatment arms significantly (p=0.0001) reduced microfilariae loads but the biannual treatment arms of ivermectin alone and ivermectin plus albendazole were the most effective regimens for clearing skin microfilariae. Ivermectin is therefore still effective in clearing microfilariae among participants in the Adansi South District of Ghana. Bi-annual treatment of ivermectin alone and ivermectin plus albendazole was found to have additional benefit in reducing microfilariae loads compared to annual treatment. However, co-administration of

ivermectin (200µg/kg) and albendazole (800µg/kg) did not have additional effect of reducing microfilariae loads.

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ABBREVIATIONS

ALB -- ALBENDAZOLE

ALT-- ALANINE AMINOTRANSFERASE

ANOVA – ANALYSIS OF VARIANCE

AST – ASPARTATE AMINOTRANSFERASE

APOC – AFRICAN PROGRAMME FOR ONCHOCERCIASIS CONTROL

CDC – CENTERS FOR DISEASE CONTROL

DEC—DIETHYLCARBAMAZINE

DEC-C—DIETHYLCARBAZINE CITRATE

DOT—DIRECTLY OBSERVED TREATMENT

GGT—GAMMA-GLUTAMYL TRANSFERASE

GPELF—GLOBAL PROGRAMME TO ELIMINATE LYMPHATIC FILARIASIS

Gm—GEOMETRIC MEAN hcG—HUMAN CHORIONIC GONADOTROPIN

IVM-- IVERMECTIN kg—KILOGRAMME

MDA—MASS DRUG ADMINISTRATION

Mf-- MICROFILARIA

OCP—ONCHOCERCIASIS CONTROL PROGRAMME

Oncho—ONCHOCERCIASIS

SIZ—SPECIAL INTERVENTION ZONES

TM—TIME POINT

WHO—WORLD HEALTH ORGANIZATION

VIT— VITAMIN

CHAPTER 1 - INTRODUCTION

1.0 BACKGROUND

Onchocerciaisis is a vector-borne nematode parasitic disease that affects approximately 37 million people worldwide, mostly in 30 countries in sub-Saharan Africa, with small foci in Latin America and Yemen (Basáñez *et al.*, 2006). The disease, commonly known as "River blindness" is a major public health problem and it is among the leading infectious parasitic causes of blindness, second only to trachoma (Thylefors *et al.*, 1995). The infective larvae (L3) of *Onchocerca volvulus* (the parasite that causes onchocerciasis) are transmitted to human by *Simulium spp*. commonly called blackflies. Over 95% of global onchocerciasis is transmitted by *Simulium damnosum*, the popular species in Africa (Townson, 1991). The vector breeds along fast flowing streams and rivers (WHO, 1995; Opoku, 2005). *Onchocerca volvulus* larvae form onchocercomas in the subcutaneous tissue and they mature into adult worms, which have an average life expectancy of about 10 years (Kale, 1998). During this period, the adult filariae can produce millions of microfilariae. These skin microfilariae are responsible for the physical manifestation of the disease. Among the physical manifestations of the disease are dermatitis, skin atrophy, and inflammation of the eye (Thylefors *et al.*, 1995; WHO, 1995).

Several programs and developments have greatly improved the onchocerciasis situation since 1974 when the Onchocerciasis Control Program (OCP) in West Africa was initiated. OCP relied exclusively on vector control in its early years. However, following the introduction of ivermectin (mectizan) in the late 1980`s, OCP transitioned to become a drug distribution program with annual distribution of ivermectin in 11 countries. OCP ended in 2002 (Thylefors *et al.*, 1995; WHO,

1995). OCP was replaced by the African Program for Onchocerciasis Control (APOC) which coordinate community directed distribution of ivermectin Mass Drug Administration (MDA) in 28 African countries (including the former OCP countries). OCP and APOC have done a good job by reducing parasite infection intensities and onchocerciasis disease rates in many endemic countries (Molyneux *et al.*, 2003).

In Ghana, onchocerciasis is endemic in 9 out of 10 administrative regions with the exception of Greater Accra Region (Taylor *et al.*, 2009a). About 3200 communities in about 60 districts are known to be endemic for onchocerciasis (Taylor *et al.*, 2009a). Two hundred and forty seven of these communities, which are in the Ashanti and Brong Ahafo regions, have been marked as special intervention zones (SIZ) and a total of about 3.4 million people are at risk of the disease (Taylor *et al.*, 2009a).

1.1 RATIONALE

In Ghana, the standard treatment of onchocerciasis is annual mass drug administration (MDA) with ivermectin at a dose of 150-200 μg/kg (Awadzi *et al.*, 2003). A standard dose of ivermectin drastically kills skin microfilariae and hinders their release by adult female *O. volvulus* (Duke, 1989). However, microfilariae production by adult female worms resumes (repopulation), which is manifested by the appearance of microfilariae in the skin of infected individuals about 3 to 6 months after ivermectin intake (WHO, 1995). Nevertheless, some female adult worms do not resume production of microfilariae for about one and half years after ivermectin intake (Duke, 1989). Studies have indicated that, several rounds of ivermectin treatment have noticeable embryogeneric effect (Winnen *et al.*, 2002; Osei-Atweneboana *et al.*, 2011). Ivermectin exposed *O. volvulus* adult worm is also known to assume a new microfilarial production level, which is reduced by 30% after each treatment round (representing a cumulative mf reduction effect) (Winnen *et al*., 2002). Thus in the presence of cumulative reduction effect on repopulation of microfilariae, there is a marked benefit of semiannual ivermectin treatment both in long and short term in reducing microfilariae prevalence than annual treatment alone (Turner *et al.*, 2013). Though Bottomley and colleagues in 2008 questioned the cumulative effect of ivermectin against microfilaria, the outcome of their study does not agree with data produced formerly in Africa (Winnen *et al.*, 2002; Bottomley *et al.*, 2008).

In the Pru and lower Black Volta basin of Ghana, where ivermectin mass treatment has been done since 1987, cases of moderately high early repopulation of microfilariae by some adult *O. volvulus* suggestive of ivermectin resistant worms have been reported (Gardon *et al.*, 2002; Plaisier *et al.*, 1997). The notable

microfilaridermia despite many rounds of ivermectin were ascribed to the nonresponse of *O. volvulus* female adult worms to ivermectin (Awadzi *et al.*, 2004a). Moreover, genetic evidence of resistance of some domestic animals to antihelmintics, including ivermectin gives cause for concern (Ardelli and Prichard, 2004; Kudzi *et al.*, 2010). Since there have been reported cases of sub-optimal response of *O. volvulus* female worms to ivermectin, monitoring of the efficacy of ivermectin in oncho-endemic communities where ivermectin-based control programs has been practiced for at least 10 years is justifiable (Awadzi *et al.*, 2004b). Furthermore, WHO has recommended the need to monitor the development of sub-optimal response to ivermectin in oncho-endemic communities where ivermectin control programe is being practiced (WHO, 1995).

Albendazole has also been proven to be an antifilarial drug (Jayakody *et al.*, 1993; Ismail *et al.*, 1998; Jayakody *et al.*, 1993; Ottesen *et al.*, 1999), with profound effect on all intra-uterine stages of *O. volvulus* manifested as partial suppression of skin microfilaria counts for at least one year (Awadzi *et al.*, 1995a). Combination therapy of albendazole at a dose of 400mg and ivermectin at a dose of 200μ/kg failed to show significant microfilariae reduction compared with ivermectin alone (Awadzi *et al.*, 1995a; Awadzi *et al.*, 2003). Nevertheless some studies have supported the need for combination therapy with the notion that drug combination therapy in filarial elimination programs might help to prevent the development of resistance of *O. volvulus* to ivermectin compared to the individual drugs (Ismail *et al.*, 1998). Ivermectin and albendazole are very safe and highly effective antifilarial drugs when given singly or in combination (Makunde *et al.*, 2003).

There is no evidence of drug-drug interaction between albendazole and ivermectin (Awadzi *et al.*, 2003). Against this background, there is the need to study higher doses and treatment frequencies of ivermectin and albendazole.

1.2 AIM

To assess the microfilaridermic efficacy of ivermectin alone and ivermectin plus albendazole against *O. volvulus* in Adansi South District of Ghana.

1.2.1 Specific objectives

1) To assess the level of endemicity of *O. volvulus* infection by determining the prevalence of onchocercal nodules and microfilariae among inhabitants in Adansi South District.
2) To compare the microfilaridermic efficacy of ivermectin plus albendazole given annually and semi-annually.
3) To compare the microfilaridermic efficacy of ivermectin alone given annually and semi-annually against *O. volvulus*.

CHAPTER 2 – LITERATURE REVIEW

2.0 HOST, PARASITE AND VECTOR DYNAMICS

Human onchocerciasis, caused by *Onchocerca volvulus* is a filarial nematode parasitic disease leading to ocular and cutaneous pathology as well as increasing host mortality (Turner *et al*., 2013). The World Health Organization Expert Committee on Onchocerciasis in 1995 estimated that, over 120 million people lived in areas where this infection was endemic (WHO, 1995). It was estimated that 500,000 and 270,000 people globally experienced secondary visual impairment and blindness respectively (WHO, 2001). Eleven Sub-Saharan West African countries like Liberia, Ghana and Mali are among nations with the highest historical prevalence of onchocerciasis (WHO, 1995).

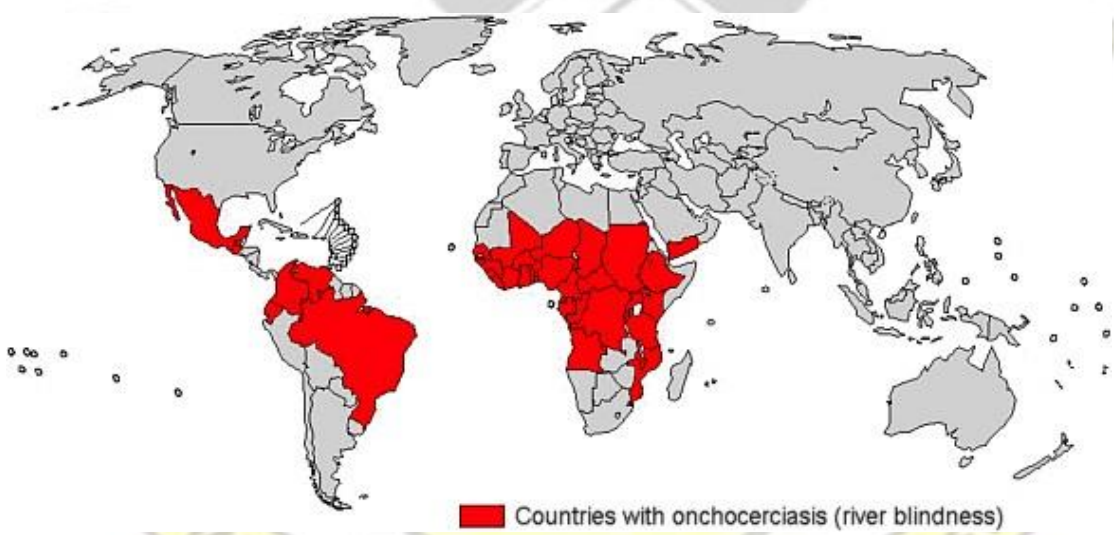


Figure 1: Global epidemiological distribution of *Onchocerca volvulus* infection.

(Source: WHO, 1995)

Human beings are the definitive host of *Onchocerca volvulus* and no animal reservoirs have been found (Awadzi *et al.*, 1995a). Human hosts different stages of the parasite, including the infective larvae, the migrating and developing pre-adult

forms, the male and female worms and the microfilariae (Awadzi *et al.*, 1995a; Krueger, 2006). In humans the adult worms are commonly found in subcutaneous nodule (onchocermata) (Awadzi and Gilles, 1992). It is normal for about 15% of individuals to host about 80% of helminth parasites in endemic human communities (Greene, 1992). Evidence shows that, there are 2 forms of onchocerciasis in West Africa: Onchcerciasis of the savannah regions and that of the forest zones (Duke and Anderson, 1972; Bryceson *et al.*, 1976). In 1972, Duke and Anderson showed differences in pathogenicity in the savanna and forest strains of *O. vovulvus.* They showed that, the microfilariae taken from patients from savanna regions produced more keratitis in the eyes of rabbits than microfilariae taken from patients in the forest zones (Duke and Anderson, 1972). *O. volvulus* has an endosymbiont bacterium *wolbachia* which has been found to be essential for the parasite fertility and survival (Taylor *et al.*, 2009b).

Simulium damnosum, which is in the Dipteran taxonomic family *Simulidae* is the only vectors of human onchocerciasis in West Africa (Boakye *et al.*, 1998). In Latin America, *S. orchraceum, S. exiguum, S. metallicum* and *S. guianeuse* are the main vectors in Mexico and Guatemala, northern and southern Venezuela and Brazil respectively (Lainson *et al.*, 2005). Blackfly biting activities which occur mostly in the morning and afternoon are affected by factors such as light intensity, clouds, seasons and temperature (Noblet, 1976; Opoku, 2005). The higher biting densities in the morning are due to the stimulating effect of the morning sunlight after inactivity in the night and a general lull in biting activities in the afternoon due to high temperature conditions of about 32^0 C (Opoku, 2005). Interactions between parasites and vectors are believed to contribute to the epidemiology patterns in vector borne infections such as onchocersiasis (Basáñez *et al.*, 2009).

Basanez and colleagues have further suggested that, the possible co-evolution of the *Onchocerca-Simulium* complex may give rise to local adaptations with the potential to stabilize the infections (Basáñez *et al.*, 2009). Studies have also shown that, the monthly onchocerciasis transmission potential, which is a basic index for assessing the disease transmission by the vectors is usually higher in the rainy season than in the dry season (Cheke *et al.*, 1992; Opoku, 2005). However, other studies have also shown the transmission potential is rather higher in the dry season than in the rainy season (Cheke *et al.*, 1992; Achukwi *et al.*, 2000).

2.1 LIFE CYCLE OF ONCHOCERCA VOLVULUS

Onchocerca volvulus has a 5-stage life cycle (Blacklock, 1927). Its infections occur when an infected blackfly introduces third stage larvae onto the skin of the human host. The larvae migrate to the subcutaneous tissue where it develops into adult worm, which normally lives in nodules in subcutaneous connective tissues (Blacklock, 1927). Nodules can habour more than one male and female worms.

Female worms measure 33 to 50 centimeters (cm) in length and 270 to 400µm in diameter while males measure 19 to 42 millimeters (mm) by 13 to 210µm (Little *et al*., 2004b). The female worms are capable of producing unsheathed microfilariae (mf) for approximately 9 years. Microfilariae have a life span of about 2years and they measure 220 to 360µm by 5 to 9µm (Blacklock, 1927). Typically, mf can be found in the skin and in the lymphatic of the connective tissue but occasionally they can be found in peripheral blood, urine and sputum (Blacklock, 1927). During a blood meal, the blackfly ingest microfilaria, which migrate through the midgut, to the hemocoel and then through the thoracic muscles. In the thoracic muscles, the microfilaria develop to first stage larvae (L1) and subsequently, it develops to the

infective larvae (L3). The infective larvae migrate to the proboscis of the blackfly, which can infect other humans during a blood meal (Blacklock, 1927).

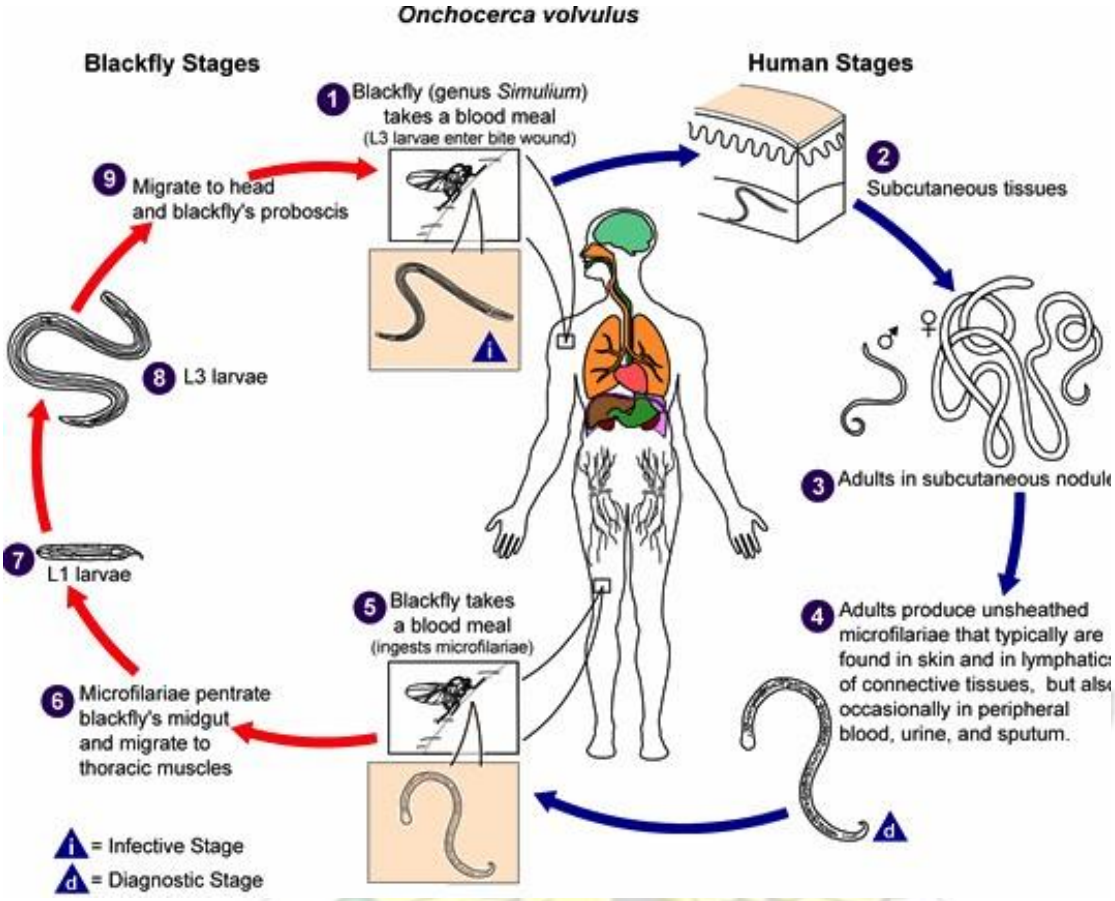


Figure 2: Digrammatic presentation of the life cycle of *Onchocerca volvulus*.

(source : Centre for disease control (CDC), http://dpd.cdcgov/dpdx)

2.2 CLINICAL MANIFESTATIONS OF ONCHOCERCIASIS

Symptoms of onchocerciasis may either be symptomatic or asymptomatic (Egbert *et al.*, 2005). The symptoms of this disease usually indicate the stage of the development of the parasite and the immunological response of the host, which is usually caused by the inflammatory response to dead or dying microfilariae (Hall and Pearlman, 1999). Individuals with onchocerciasis usually show one or more of these three general manifestations: (i) Onchocercal dermatitis (ii) Ocular onchocerciasis and or (iii) Sub-cutaneous bumps or nodules (onchocercomata), with

the most serious manifestation which include eye lesions that can lead to blindness (Hall and Pearlman, 1999). Onchocerciasis has been found to be associated with musculoskeletal pain, reduced body mass and decreased work productivity (Basáñez *et al.*, 2006). This may be due to the fact that microfilariae can invade many tissues and organs. Severe *Onchocerca volvulus* infection has also been suspected to be involved in the onset of epilepsy (Boussinesq *et al*., 2002).

2.3 SUBCUTANEOUS NODULES (ONCHOCERMATA)

The least severe clinical manifestation of onchocersiasis is the occurrence of onchocerca nodules in the subcutaneous tissues of infected individuals (Awadzi and Gilles, 1992). The male and female worms are enclosed in the nodules within which fertilization occurs (Basáñez *et al.*, 2006). Onchocerca nodules are usually scattered around the body over bony areas (Kale, 1998).

2.4 ONCHOCERCAL DERMATITIS

About 30% of the populations in oncho-endemic areas have onchocercal dermatitis (Hagan, 1998). Oncho-dermatitis is therefore the most reported symptom of the disease (Hagan, 1998). Most people with oncho-dermatitis experience severe itching of the skin, which is common in all age-groups (Hagan, 1998; Hailu *et al.*, 2002). Onchocercal dermatitis may progress to papular rashes, which is also known as acute papular dermatitis. Acute papular dermatitis is presented as pruritic papules, which often develop into pustules or vesicles. Papular dermatitis often affects the face, the trunk and the extremities (Enk, 2006). Acute papular dermatitis can progress to chronic papular dermatitis, which may result in hyperpigmentation and thickening of the skin (Murdoch *et al.*, 1993). Further physical deterioration leads to lichenified onchodermatitis which is popularly known as lizard skin or *sowda* (Okoye and

Onwuliri, 2007). *Sowda* is associated with a delayed hypersentivity immune response, usually observed in patients with low microfilariae loads (Enk, 2006). Advanced-stages of onchocercal dermatitis are characterized by loss of elasticity and depigmentation of the skin, popularly known as leopard skin (Murdoch *et al.*, 1993; Okoye and Onwuliri, 2007).

2.5 OCULAR ONCHOCERCIASIS

Ocular onchocerciasis occurs as a result of inflammatory reactions due to the presence of microfilariae in the eye (Egbert *et al.*, 2005). The inflammatory responses which are triggered by the death of mf, can involve all the eye tissues except the lens (Basáñez *et al.*, 2006; Taylor *et al.*, 2010). Punctate keratitis which occur as a result of ocular onchocerciasis is transient and reversible with treatment whereas sclerosing keratitis, iriocyclitis and inflammation in the anterior chamber and epithelium result from long term infection (Egbert *et al.*, 2005; Taylor *et al.*, 2010). Blindness may occur as a result of immunological reactions resulting from the death of mf (Taylor *et al.*, 2010). Onchocerciasis blindness is more likely in Africa than in the Latin America (Basáñez *et al.*, 2009) and this has been attributed to existence of possible biological variants (Kale, 1998; Murdoch *et al.*, 2002).

2.6 GLOBAL CONTROL OF ONCHOCERCIASIS

Global control of onchocerciasis were implemented by Non-Governmental Organizations (NGOs) and the Onchocerciasis Control Programme (OCP) in West Africa. The OCP was started in 1974. The programme initially was created to control the *Simulium* vector through aerial larviciding, which was directed against the aquatic stages of the vector. In 1987, when ivermectin was registered for human use

against onchocerciasis, OCP was transitioned to be a drug administration programme with annual distribution of ivermectin in 11 countries.

The programme was started in Benin, Burkina Faso, Cote d`Ivoire, Ghana, Mali, Niger and Togo. Merck and Co. Inc. took the decision to donate ivermectin to eliminate onchocerciasis as a public health problem (Thylefors *et al.*, 1995). It is estimated that about 600,000 cases of blindness were prevented, 18 million children born in onchocerciasis endemic areas were freed from the risk of blindness and 25 million hectares of land was made safe for human resettlement (Thylefors *et al.*, 1995; WHO, 1995).

The African Programme for Onchocerciasis Control (APOC) was launched in 1995 to target the 19-onchocerciasis endemic countries in Africa not covered by OCP. The strategy of APOC involved the establishment of community-directed annual mass administration of ivermectin for all those aged five years and older (Molyneux *et al.*, 2003). APOC, which was initially conceived to end in 2007, and subsequently in 2015, would be succeeded by the Programme for the Elimination of Neglected Diseases in Africa (PENDA) with a wider mandate to tackle all the five preventive chemotherapy diseases (River blindness, elephantiasis, trachoma, bilharzia, and soil transmitted helminthiasis). One of the specific goals of PENDA is to eliminate onchocerciasis by year 2025 (WHO/APOC, 2013).

2.7 CHEMOTHERAPEUTIC APPROACHES TO ONCHOCERCIASIS

Ivermectin is administered annually or semi-annually to people living in onchoendermic communities of ages five years or older, excluding pregnant women and mothers breastfeeding a baby younger than one week (Collins *et al.*, 1992; Boussinesq *et al.*, 1997; Tielsch and Beeche, 2004). Ivermectin is currently the drug of choice for the control of onchocerciasis (Hoerauf *et al.*, 2003). However, several drugs have been proposed as possible chemotherapy to eliminate onchocercaciasis (Francis *et al.*, 1985; Poltera *et al.*, 1991; Molyneux, 1995; Tagboto and Townson, 1996; Hoerauf *et al.*, 2003).

2.7.1 Activities of ivermectin in onchocerciasis control

Ivermectin is a potent microfilaricide, which causes marked reduction of microfilariae loads in a short period after treatment, followed by a steady repopulation of the mf (Alley *et al.*, 1994). The recommended dosage for most control programs is 150-200 μg/kg of body weight (Awadzi *et al.*, 1995c). Although ivermectin has been shown to interfere with adult female worm ability to produce microfilariae (Duke *et al.*, 1992), it neither kills nor permanently sterilizes the adult worm (Awadzi *et al.*, 1995c). Generally, ivermectin is well tolerated, although there are adverse effects associated with it, 1 to 2 days after treatment (Taylor *et al.*, 2010). However, this infrequent, transient and unusual mild adverse effects such as pruritus, urticaria, dermatitis, fever, myalgia and edematous swelling of the limbs and face corresponds with microfilariae status of the individual (Taylor *et al.*, 2010). Meanwhile, a major side effect like encephalitis arises when individuals with *O. volvulus* and *Loa loa* co-infections are treated with ivermectin (Taylor *et al.*, 2010). Ivermectin is an avermectin compound of macro cyclic lactones derived from the

bacterium *Streptomyces avermitilis* (Geary, 2005). The mechanism by which ivermectin kills microfilariae is not known with certainty, but the drug interferes with glutamate- gated ion channels that affect parasite contractility (Moreno *et al.,* 1983).

2.7.2 Activities of albendazole in onchocerciasis control

Albendazole has no microfilaricidal activity, but it is toxic to all intra-uterine stages of *O. volvulus,* possessing important chemosteriant properties, which are enhanced by administration with fatty breakfast (Awadzi *et al.,* 1995a). Albendazole causes degenerative alterations in the integument and intestinal cells of the worm by binding to the colchicine-sensitive site of tubulin, thus inhibiting its polymerization or assembly into microtubules (Horton, 2002). The loss of cytoplasmic microtubules leads to impaired uptake of glucose by larval and adult stages of the parasite and depletes glycogen stores. Degenerative changes in endoplasmic reticulum and mitochondria of the germinal layer, and the subsequent release of lysosomal enzymes result in decreased production of adenosine triphosphate, which is the source of energy required for survival of the helminth. Due to diminished energy production, the parasite is immobilized and eventually dies (Horton, 2002).

2.7.3 Activity of Diethylcarbamazine (DEC) in onchocerciasis control

In the 1940`s diethylcarbamazine, also known as diethylcarbamazine citrate (DECC) was the approved microfilaricidal drug for the treatment of filariasis (Taylor *et al.,* 2010). DEC produces severe reactions (including Mazzoti reactions) when used in onchocerciasis treatment compared to ivermectin (Awadzi and Gilles, 1992). However, the former has moderate macrofilaricidal effect. DEC is therefore used in

lymphatic filariasis control programs in non oncho-endemic regions (Pfarr and Hoerauf, 2006).

2.7.4 Activity of Suramin in Onchocerciasis control

Two years post treatment with suramin almost totally eliminates both ocular and skin microfilariae, though at a physiological cost (renal malfunction) to some patients (Awadzi and Gilles, 1992). Also examination of subcutaneous nodules of these patients revealed embryostatic effects for 6 weeks. Suramin has lethal effects on male and female worms at 3 and 6 months after treatment respectively (Awadzi and Gilles, 1992). It is therefore one of the few officially recognized and highly effective macrofilaricides (Thylefors and Rolland, 1979). However, treatment with suramin should be strictly under supervision, usually in a hospital setting. It is therefore considered to be too toxic and as such cannot be used for mass drug administration (Thylefors and Rolland, 1979).

2.7.5 Activity of Moxidectin in onchocerciasis control

Moxidectin is structurally similar to ivermectin and it has the same method of action and binds to the same site as ivermectin (Taylor *et al.*, 2010). It is also highly lightly effective microfilaricide with longer half-life compared to ivermectin (Cotreau *et al.*, 2003).

2.7.6 Activity of Antibiotics against oncohcerciasis

A novel approach using antibiotics to target the endosymbiont *wolbachia* of *O. volvulus* has been shown to be effective (Pfarr and Hoerauf, 2006). The principle for this approach stem from the earlier findings in both animal and human trials where depletion of the *wolbachia* endobacteria in adult filarial worms following treatment

with some antibiotitics like doxycycline precede female worm sterility and worm death (Hoerauf *et al.*, 2003; Debrah *et al.*, 2006; Hoerauf *et al.*, 2008).

CHAPTER 3 – MATERIALS AND METHODS

3.0 STUDY AREA AND POPULATION

This study was conducted in the Adansi South District of the Ashanti Region of Ghana in 40 onchocerciasis-endemic communities. The district is bordered at the south by the Central Region, the east by the Eastern Region and by Adansi North, Obuasi Municipal, and Bosome Freho District at northeast, northwest, and southwest respectively. The district capital is New Edubiase. The major rivers found in these communities are River Offin and River Pra. These are fast flowing rivers, which are ideal sites for breeding the vector. The inhabitants are mainly cocoa and rice farmers. The communities involved in this study were closer to these rivers. According to mapping by the Ghana Health Service (GHS), the district is considered as hyper-endemic for onchocerciasis infection. Ivermectin mass drug administration has been ongoing for at least 10 years. The district has a diversity of ethnic groups such as Krobos, Ewes, Fantes, Akuapem, Akyems and the indigenous Ashantis. The district covers an area of 1380 kilometers square (Ghana, Statistical Service, 2010 population census).

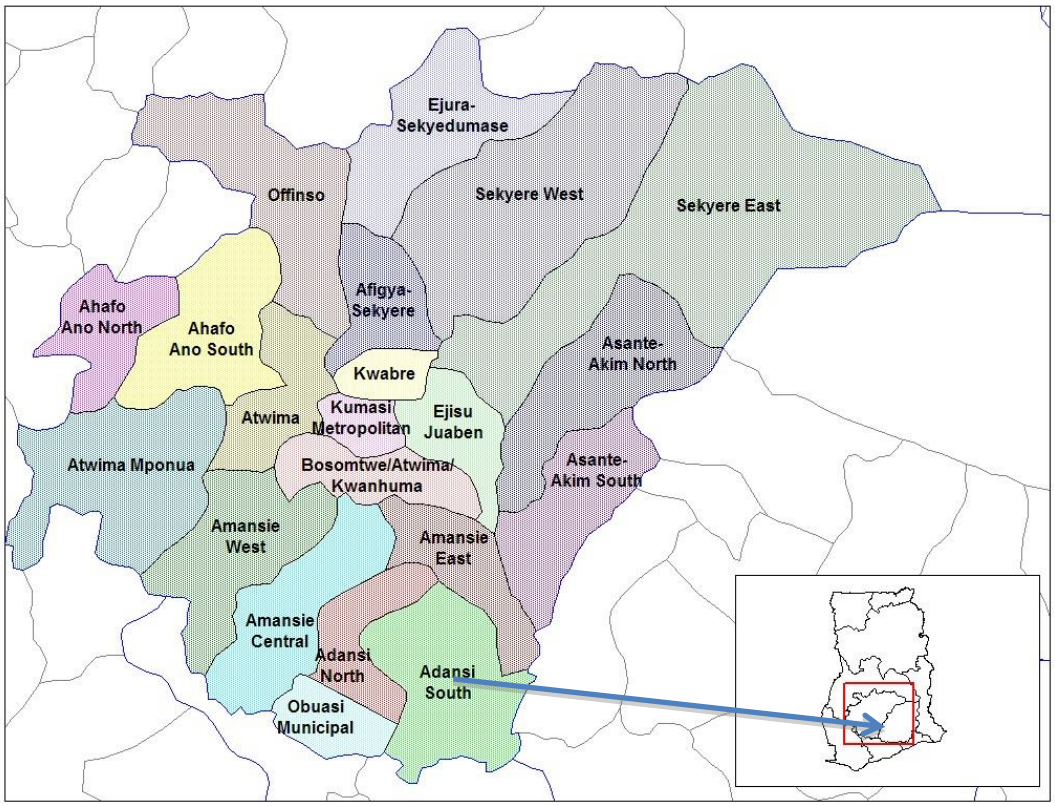


Figure 3: Map of study Area : Adansi South District.

Source: (Ghana, Statistical Service, 2010 population census)

3.1 ETHICAL APPROVAL

This study was approved by the Committee on Human Research, Publication, and Ethics of the School of Medical Sciences of the Kwame Nkrumah University of Science and Technology (KNUST). This study was conducted in accordance with the principles of the Helsinki Declaration of 1964 (as revised in 1983, 2000 and 2004). Additional permission was sought from the Akrofuom and New Edubiase sub-district Health directorates of the Adansi South District of Ashanti Region.

Chiefs, opinion leaders, and inhabitants of participating communities were consulted. Now the purpose and procedures of the study were explained to the study participants in their local language. Written informed consent was obtained from all participants either by thumb printing or signing of signature.

3.2 STUDY DESIGN

The study was an open-labelled randomized clinical trial where volunteers and investigators recruiting patients and administering the drugs knew the drugs being used in the study.

3.3 STUDY PROCEDURE

3.3.1 Enrolment of study volunteers

Research team contacted the Adansi South District Health Directorate before entering the endemic communities. Research team explained the entire protocol of the study to the directorate. The disease control officer of the district led the research team to meet each of the community health volunteers (CHV) of the onchocerciasis endemic villages. With the help of the CHV, the research team was introduced to the chiefs and elders of each community. Research procedures and study protocol were then explained to the chiefs and elders of each individual community in the local language. A day was then scheduled for community surveys. On the scheduled day, gong gong was beat for the research team to meet the entire community members. The research team, upon meeting the community members explained the study protocols and procedure to them in the local dialect.

Community members were allowed to ask questions before survey begun. Study volunteers were allowed to sign the informed consent form to indicate their willingness to participate in the study. Volunteers identified with one or more

accessible nodules via palpation underwent physical examinations before a piece of skin snipped from each buttock using a sclera punch as described in section to determine the presence of skin mf. Ten milliliters of peripheral venous blood were taken from individual found to be positive for skin mf to perform liver transaminase aspartate aminotransferase (AST), alanine aminotransferase(ALT), glutamyl transferase (γGT) and creatinine tests. Urine analysis was also performed prior to final enrolment of volunteers on fresh urine.

Plate 1: Researchers explaining study protocols to a study community

3.3.2 Inclusion criteria for enrolment of volunteers

The inclusion criteria were as follows:

i) Male and female from 18 to 60 years residing in Adansi South District.

ii) Presence of onchocercal nodules. iii) Presence of microfilaria in skin snip.

iv) Minimum body weight of 40kg which suggest that the individual is malnourished.

v) Normal hepatic profiles vi) Willingness to participate in the study as evidenced by signing or thumb printing of the informed consent document.

3.3.3 Exclusion criteria for enrollment of volunteers

The exclusion criteria were as follows:

i) Female volunteers who tested positive for urine pregnancy test.
ii) Breastfeeding volunteers.
iii) Patients suffering from medical conditions requiring long term medications such as hypertension and diabetes.
iv) Significant glycosuria or proteinuria.

3.3.4 Examination for onchocercal nodules

Characteristics of onchocercal nodules were described to all volunteers as hard, mobile and most often round nodules favouring bony-prominences outside the inguinal and cervical regions. The presence of subcutaneous nodules was determined according to the standard World Health Organization (WHO) protocol (WHO, 1995). Participants were asked if they were aware of any nodules present prior to the standard physical assessment for nodules. The body of each volunteer was examined by an experience scientist systematically following a standardized routine that gave particular attention to the bony regions by palpation.

3.4 LABORATORY PROCEDURE

3.4.1 Skin biopsy and microfilariae count

Volunteers with palpable nodules were skin-snipped during recruitment, 6 and 18 months-time points. Skin microfilariae levels of study volunteers were assessed during recruitment, 6 and 18 months by examining skin biopsies taken from the left and right iliac crest at the buttocks to determine the number of microfilariae per milligrams of skin. Skin biopsies are the "gold standard" in detecting the presence of *O. volvulus* microfilariae infections in infected individuals. Even though it is invasive, skin biopsies are in line with the WHO decision on the need for surveillance methods to be highly specific (WHO, 1995).

About 100µl of physiological saline (0.9%) was pipetted into a 96-well round bottom microtitre plate that had been labelled with volunteers identification numbers. The skin of the left and right iliac crest were cleansed using 70% alcohol and then allowed to dry. Sterilized Holth-corneoscleral punches were used to take bloodless skin biopsies from both left and right iliac crest of volunteers. The snips were immersed into the normal saline pipetted into the microtitre plates. To exclude any bacterial infection the snipped areas were dressed by covering with antibacterial padded plasters. The punches were sterilized using 10% mucocit solution for 5-10 minutes according to the manufacturers protocol. The wells of the plates were covered with adhesive tapes to prevent evaporation and spillage of the contents during transportation from the communities to the New Edubiase Government Hospital laboratory. The snips were incubated overnight at room temperature to allow the emergence of microfilariae into the saline solution.

Solution in each well was thoroughly mixed before pipetting onto a clean glass slide for microscopic examination under a light microscope using the 10x objective lens with the condenser iris closed sufficiently for good contrast. The observed microfilariae were counted with a tally counter and the results recorded. Each skin snip was blotted and weighed using OHAUS® Adventure Pro analytical electronic balance and the number of mf from each biopsy determined as mf per milligram (mf/mg) of skin. Plate 2 is the complete set up for skin biopsy.

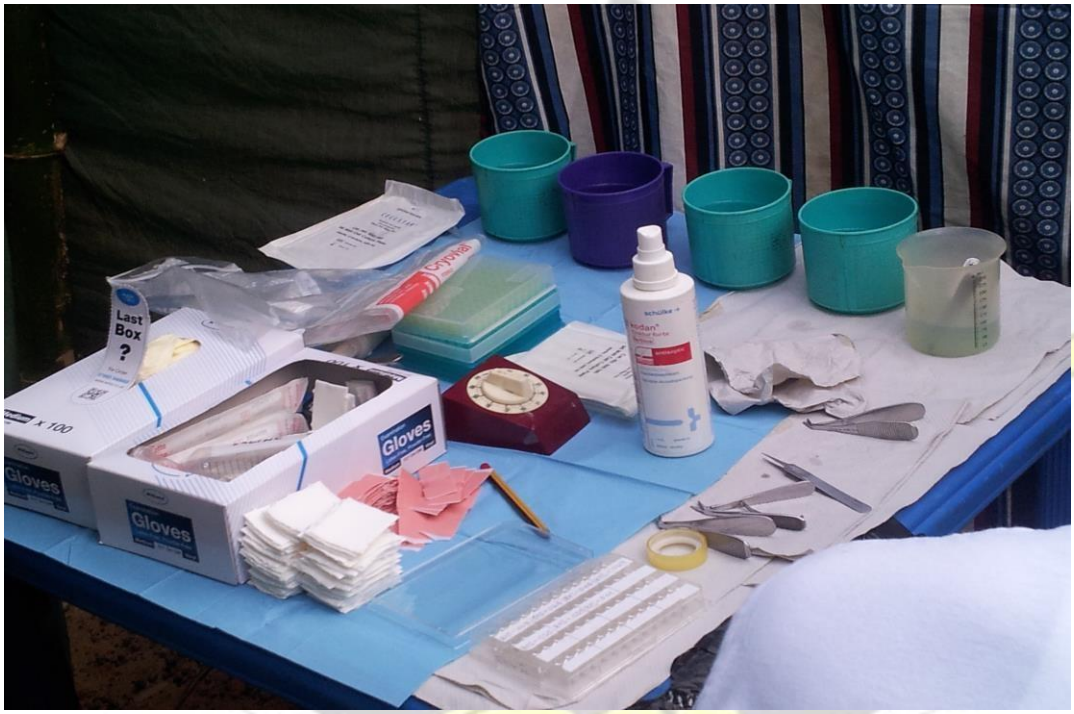


Plate 2: Complete set up for skin biopsy.

Plate 3: Examination of skin snip in the laboratory for skin microfilaria.

3.4.2 Assessment of renal and hepatic profiles of study volunteers

Clinical bichochemistry tests were performed to assess volunteers' kidney and liver functions using CHEM 7 (semi-automated) and VITALAB SELECTRA JUNIOR (automated) biochemistry analyzers. Ten milliliters of blood were collected from each volunteer and then centrifuged to separate plasma from blood cells. About one milliliters (ml) of each volunteer's plasma was pipetted into a 1.8 ml eppendorf tube bearing the volunteers identification numbers, which were used to determine kidney and liver enzymes levels of study volunteers. Liver transaminases aspartate aminotransferase (AST), alanine aminotransferase(ALT), glutamyl transferase (γGT) and creatinine were the enzymes that were checked to monitor the liver and kidney functions of study volunteers.

3.4.3 Urine Chemistry analysis and pregnancy test

Fresh urine in a clean and dry container was taken from participants who were found to be positive for both nodules and mf. Urine chemistry test was then performed using combi urine rapid test strip in order to determine liver and kidney functioning status as well as urine sugar levels of each participant. In female volunteers, pregnancy test (urine dip stick) was also done using HCG Pregnancy test strip at every time point before drug administration.

3.5 SOURCE OF DRUGS USED FOR THE STUDY

Global Program to Eradicate Lymphatic Filariasis (GPELF) in Ghana provided the drugs for the study. These drugs were collected and stored in Kumasi Center for Collaborative Research (KCCR) into Tropical Medicine cold room, before transporting to the field for administration.

3.6 PATIENT RANDOMIZATION AND TREATMENT ARMS

A total of 272 participants were randomized into 4 treatment arms (68 participants in each arm) as described below

i) Treatment arm 1: The comparator (standard treatment). Ivermectin 200µg/kg body weight given at 0 and 12 months plus vitamin C pills at 6 months. ii) Treatment arm 2: Ivermectin 200µg/kg body weight given at 0, 6 and 12 months.

iii) Treatment arm 3: Ivermectin 200µg/kg body weight plus albendazole 800mg (regardless of body weight) given at 0 and 12 months plus vitamin C pills at 6 months.

iv) Treatment arm 4: Ivermectin 200µg/kg body weight plus albendazole 800mg (regardless of body weight) given at 0, 6, and 12 months.

3.7 TREATMENT PROCEDURES

Volunteers were given study medications under direct observed treatment (DOT) every six months by trial clinicians in the study communities. Treated volunteers at each time point were monitored for seven days by trial clinicians and researchers for adverse events that may possibly arise in the course of the treatment. Participants were actively and passively monitored for the first three days and the last four days after each treatment respectively. Volunteers in the annual treatment arms received vitamin C at 6 months. This kept every volunteer engaged at 6 months interval and the psychological benefit of receiving a pill that could be of benefit for them. There was no downside for volunteers not receiving drugs at alltime points since every volunteer received the standard treatment of ivermectin.

Plate 4: Patient (in the middle) being treated

Plate 5: Patient (sitting) being examined for an adverse event.

3.8 STATISTICAL ANALYSES

To estimate the power for this study, a simulation analysis was performed, assuming a dependency between the fertility rates of different female worms in the same person as estimated from available data. Assuming 1.5 worms per nodule and 3 nodules per person (4.5 worms per person) as well as fertility rates of 30% for the standard therapy and 20%, 15% and 10% for the experimental treatments of alone ivermectin biannually, ivermectin plus low dose albendazole biannually and ivermectin plus high dose albendazole biannually respectively. A power of 97% to detect a difference between the worst and the best and a power of 80% for the difference between the worst and the second best treatment regimen when choosing 52 participants per treatment arm. Previous studies revealed a 20% loss to follow-up 20 months after treatment, therefore a drop-out rate of 30% was calculated for this study with the last observation 36 months after treatment resulting in 68 participants for each treatment group. Statistical analyses were done using StatView® and Microsoft excel software programs. Descriptive statistics were used to obtain general descriptive information such as the geometric mean (Gm) and standard deviations from the data. The geometric mean of the mf from paired skin biopsies from each patient was calculated and was used as a measure of intensity of infection. Chi-square test was used to compare two qualitative proportions or groups. One-way ANOVA (Analyses of Variance) was used to test group means and standard deviations of demographic data. For non-parametric data set, analyses were done using Wilcoxon Signed Rank test for paired variables that were not normally distributed and Kruskal Wallis was used to compare more than two quantitative variables. A p-value of less than 0.05 ($p<0.05$) was

considered statistically significant.

CHAPTER 4 – RESULTS

4.0 DEMOGRAPHIC DATA OF THE STUDY VOLUNTEERS

A total of 2,326 volunteers from 40 villages were recruited for this study. All volunteers were examined for the presence of onchocercal nodule (onchocercoma). Out of the 2,326 volunteers, 58.2% were males with a mean age of 38.7 years and age range of 18 to 60 years and 41.8% were females with a mean age of 37.2 years and age range of 18 to 60 years (Table 1). There was no significant difference between the mean ages for both sexes (p=0.189). The overall mean age was 37.6 years (Table 1).

Table 1 Demographic data of total study volunteers

Gender of study volunteers	**Number of volunteers**	**Age (Mean ± SD)/years**
Male	1354 (58.2%)	38.7 ± 12.6
Female	972 (41.8%)	37.2 ± 12.2
Total	2326 (100%)	37.6 ± 12.4

4.1 NODULES AND MICORFILARIAE ASSESSMENT

Table 2 shows the prevalence of nodules and microfilariae of the study population. A total of 982 out of the 2,326 volunteers who were examined from 40 communities in the Adansi South district had palpable onchocercomas. This represents 42.2% infection. Also, skin snip examination revealed that 368, representing 37.5% of the volunteers who had palpable onchocercomas, were microfilaridermic (Table 2). Also, 589 (43.5%) of the total male volunteers who were examined had at least one

palpable onchocercoma (nodule). However, 244 (41.4%) of male volunteers who had at least one palpable nodule were positive for skin microfilariae, whilst 393 (40.4%) and 124 (31.6%) of the female volunteers had at least one palpable onchocercoma and skin microfilaria, respectively.

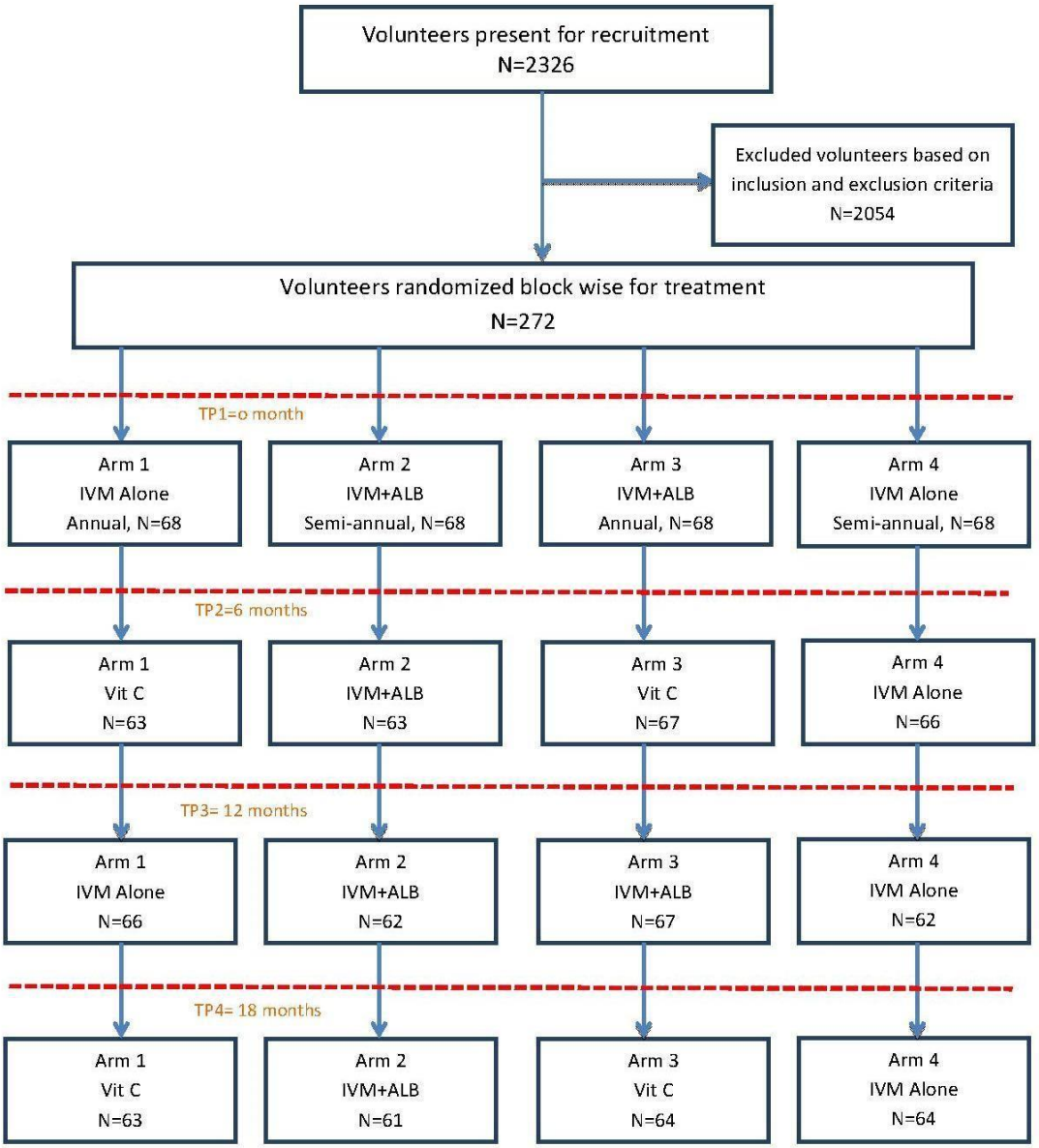
Table 2 Prevalence of *O. volvulus* among study volunteers.

	Number of volunteers examined	Number of Positive volunteers	Prevalence (%)
Volunteers with nodules	**2326**	**982**	**42.2**
Volunteers with mf	**982**	**368**	**37.5**
Male volunteers with nodules	**1354**	**589**	**43.5**
Female volunteers with nodules	**972**	**393**	**40.4**
Male volunteers with mf	**589**	**244**	**41.4**
Female volunteers with mf	**393**	**124**	**31.6**

4.2 VOLUNTEER PARTICIPATION, TREATMENT AND DROPOUTS

Of the 2326 volunteers examined, 272 met the inclusion criteria and were enrolled into the study and subsequently randomized into four treatment arms: (1) ivermectin alone once a year (2) ivermectin alone twice a year (3) ivermectin and albendazole once a year and (4) ivermectin and albendazole twice a year treatment arms as shown in figure 3 below. Sixty-eight randomized volunteers were assigned to each treatment arm according to the sample size calculation. Two hundred and forty one volunteers completed the treatment at all the four treatment time-points.

Thirteen (4.8%), 15 (5.5%) and 22 (8.1%) volunteers could not receive treatment at 6 months, 12 months and 18 months respectively, due to a variety of reasons, such as patient refusing to take treatment, travelling out of town, etc (Figure 4).



TP-TIME POINT.

Figure 4: Flow chart of patients participation.

4.3 DEMOGRAPHIC DATA OF RANDOMISED VOLUNTEERS

As shown in Table 3 below, there were no statistical difference in the age and weight of volunteers in all the four treatment arms (P=0.3004 and 0.4374 respectively). Also of the randomised volunteers, 180 (67%) were males and 92 (33%) were females.

Table 3 Demographic data of treated volunteers

Study volunteers	IVM alone annually	IVM alone semiannually	IVM and ALB annually	IVM and ALB semiannually	Total	P-Value
Number of volunteers at treatment start	68	68	68	68	272	
Number of male volunteers	40	44	53	43	180 (67%)	
Number of female volunteers	28	24	17	23	92 (33%)	
Age in years (Mean ± SD)	43± 9	40 ± 10	41 ± 11	41 ± 10	41 ± 10	**0.3004**◆
Weight in kilograms (Mean±SD)	56 ± 9	58 ± 8	58 ± 8	57 ± 9	57 ± 9	**0.4374**◆

◆ - ANOVA; SD = Standard Deviation

4.4 TYPES OF ADVERSE EVENTS EXPERIENCED BY VOLUNTEERS

Table 4 shows the types of adverse events at each treatment time point. Ivermectin alone and ivermectin taken in combination with albendazole were all tolerated with no serious adverse events. Adverse events reported included swollen limbs, swollen face, ocular reactions, cutaneous reaction (itching), cutaneous reaction (rash), nausea and dizziness. A total of 398, 69, and 44 different minor adverse events were reported at 0, 6 and 12 months treatment time points, respectively. At

all treatment time points, some participants reported more than one adverse reaction. Cutaneous reaction was the most reported adverse reaction at the treatment time points. Severe symptomatic postural hypotension and dyspnoea were not reported at all (Table 4).

Table 4 Types of adverse events at treatment time points among treated volunteers

Types of adverse events	0 Months	6 Months	12 Months
Pain condition	52	10	8

Headache	22	13	2
Gland reaction	2	0	0
Fever	27	2	2
Swelling of limb	59	1	1
Swelling of face	7	1	1
Swelling of other body regions apart from the face and limbs	7	0	0
Ocular reactions	4	3	2
Cutaneous reaction (Itch)	126	20	16
Cutaneous reaction (Rash)	29	3	6
Severe symptomatic postural hypotension	0	0	0
Dyspnoea	0	0	0
Anorexia	5	1	0
Nausea	2	0	0
Vomiting	2	0	0
Dizziness	6	1	0
Insomnia	2	0	0
Other advere events	44	14	6
TOTAL	398	69	44

4.5 ADVERSE EVENTS EXPERIENCED BY VOLUNTEERS IN EACH TREATMENT ARM

As shown in Table 5, a total of 186 (68%) volunteers reported adverse events at the first treatment time point. This time point recorded the most adverse events. However, the number of volunteers who reported adverse events decreased as the number of treatment rounds increased in all the treatment arms. Thus, 186 (68%)

reported minor adverse events during the first treatment time point whiles 37 representing 14% reported adverse events at 12 months treatment time point.

Table 5 Proportions of adverse events in each treatment arm at various treatment time points

Time points	IVM alone annually	IVM alone semiannually	IVM and ALB annually	IVM and ALB semi-annually	Total of adverse at treatment time points
0 month	43 (63%)	48 (71%)	50 (74%)	45 (66%)	186 (68%)
6 months	11 (17%)	15 (24%)	5 (7%)	14 (21%)	45 (17%)
12 months	9 (14%)	5 (8%)	12 (18%)	11 (18%)	37 (14%)

4.6 ADVERSE EVENTS REPORTED BY VOLUNTEERS TREATED WITH IVERMECTIN ALONE AND IVERMECTIN PLUS ALBENDAZOLE

From Table 6 below, 95 volunteers treated with ivermectin plus albendazole reported adverse events at treatment start. Ninety one volunteers treated with ivermectin alone also reported adverse events. Among volunteers who reported adverse events, there was no significant difference between the proportion of volunteers treated with either ivermectin alone or ivermectin plus albendazole at

treatment start time-point (p=0.5510). At 12-month treatment time point, a total of 37 volunteers reported adverse events. Fourteen out of these 37 volunteers were treated with ivermectin alone and the rest (23) took ivermectin plus albendazole. There was a significant difference between the proportions of volunteers treated with ivermectin alone and ivermectin plus albendazole, who reported adverse events at 12-months treatment time-point (p<0.0001) (Table 6).

Table 6 Comparison of adverse events reported by volunteers treated with ivermectin alone and ivermectin plus albendazole

Adverse Events	IVM alone	IVM and ALB	Total	P-value
Number of volunteers who reported adverse events at 0 month	91 (48.9%)	95 (51.1%)	186 (100%)	0.5510◆
Number of volunteers who reported adverse events at 12 months	14 (37.8%)	23 (62.2%)	37 (100%)	<0.0001◆

◆- Chi Square Test.

4.7 ADVERSE EVENTS REPORTED BY VOLUNTEERS TREATED WITH IVERMECTIN ALONE OR IVERMECTIN PLUS ALBENDAZOLE AND VITAMIN C

From Table 7, 45 volunteers reported adverse event at 6-month treatment time point: 16 (35.6%) received vitamin C alone and 29 (64.4 %) received ivermectin alone or ivermectin plus albendazole. There was a significant difference between the number of volunteers treated with either ivermectin alone and ivermectin plus albendazole

or vitamin C who reported adverse events at 6-months treatment timepoint ($p<0.0001$).

Table 7 Comparison of adverse events reported by volunteers treated with either ivermectin alone or ivermectin plus albendazole with those treated with vitamin C alone.

Adverse Events	VITAMIN C alone	IVM alone or IVM plus ALB	Total	P-value
Number of volunteers with Adverse events at 6 months	16 (35.6%)	29 (64.4 %)	45 (100%)	< 0.0001 ◆

◆- Chi Square Test.

4.8 MICROFILARIDERMIA BETWEEN ANNUAL AND SEMI-ANNUAL TREATMENT ARMS

Table 8 below shows the assessment of microfilaridermia of the volunteers in the annual and semi annual treatment arms. The overall number of microfilaridermic volunteers decreased from 272 (100%) to 99 (36.4%) and 39 (14.3%) at 6 and 18 months follow-ups, respectively. The two semi-annual treatment arms recorded the most reductions in the microfilaridermic volunteers (Table 8). At 18 months follow up, the least reductions in the number of volunteers who were positive for microfilariae occurred among the volunteers treated with ivermectin alone once per

year whereas the most reductions in the number of microfilaridermic volunteers occurred among volunteers treated with ivermectin alone twice per year. Comparative assessment of microfilaridermic volunteers treated with ivermectin alone annually and semi-annually at 18 months time point showed a significant difference (p=0.0055), but there was no significant difference in the microfilaridermic volunteers treated with ivermectin plus albendazole annually and semi annually (p= 0.1151) at the same 18 months time point (Table 8).

Table 8 Assessment of microfilaridermia for annual and semiannual treatment arms.

Treatment arms	**Pre-treatment mf+**	**6 months mf+**	**18 months mf+**
IVM alone annual	68 (100%)	26 (41.2%) n=63	15 (23.8%) n=62
IVM alone semiannual	68 (100%)	26 (39.3 %) n=66	4 (6.3%) n=63
P-value		**P=0.8281◆**	**P=0.0055◆**
IVM plus ALB annual	68 (100%)	28 (41.8%) n=67	12 (18.8%) n=64

IVM plus ALB semi-annual	68 (100%)	19 (30.2%) n=63	8 (13.1%) n=61
P-value		**P=0.1931◆**	**P=0.1151◆**
TOTAL	**272**	**99**	**39**

◆-Chi Square Test

4.9 MICROFILARIDERMIA OF VOLUNTEERS TREATED WITH IVERMECTIN ALONE AND IVERMECTIN PLUS ALBENDAZOLE

From Table 9, which shows the microfilaridermia of volunteers treated with ivermectin alone and ivermectin plus albendazole, there was no significant difference between the number of volunteers treated with ivermectin alone and ivermectin plus albendazole annually at both 6 and 18 months treatment time points (p=0.8943 and 0.3537, respectively). Even though there was drastic reduction in the number of microfilaridermic volunteers treated semi-annually with ivermectin alone and ivermectin plus albendazole, there was no significant difference between these treatment arms both at 6 and 18 months (p=0.8943 and 0.3537 respectively), showing that the addition of albendazole has no added effect on microfilaria depletion at 18 months after treatment onset (Table 9).

Table 9 Assessment of microfilaridermia between IVM alone and IVM plus ALB treatment arms

Treatment arms	Pretreatment mf positive	6-months mf positive	18-months mf positive
IVM alone annually	68	26 (41.3%) n=63	15 (24.2%) n=62

IVM plus ALB annually	68	28 (41.8%) n=63	12 (18.8%) n=61
P- value		**P = 0.8943◆**	**P = 0.3537◆**
IVM alone semiannually	68	26 (39.4%) n=66	4 (6.3%) n=63
IVM plus ALB semi-annually	68	19 (30.2%) n=63	8 (13.1%) n=61
P- value		**P = 0.8534◆**	**P = 0.2537◆**
TOTAL	**272**	**99 (38.2%)**	**39 (15.6%)**

◆- Chi Square Test.

4.10 MICROFILARIA LOADS OF VOLUNTEERS AT TREATMENT TIME POINTS

Table 10 shows that mf loads of volunteers who took ivermectin alone annually drastically decreased from a geometric mean of 6.12 (100%) at pre-treatment to 1.26 (21.3%) at 6 months time point. There was a significant difference (p=0.0001) in the microfilariae geometric mean loads at pre- treatment and six month time points of volunteers who took ivermectin alone annually. However, there was a marginal reduction in the microfilariae geometric mean loads of volunteers from 1.26 to 1.10 at six and 18 months time points respectively with no significant difference (p=0.29). Also, Table 10 shows drastic reductions in the microfilariae geometric mean loads from 5.27 (100%) to 1.25 (23.7%) volunteers who took ivermectin alone semi-annually at pre-treatment and 6 months time point, respectively. However, from 6 to 18 months time points, there was a marginal significant reduction from 1.25 to 1.02

respectively (p=0.002) in the microfilariae geometric mean loads in volunteers who took ivermectin alone semi-annually.

Also as shown in Table 10, volunteers who were treated with ivermectin and albendazole annually recorded drastic reduction in mf geometric mean loads from 6.32 (100%) to 1.33 (21.04%) at pre-treatment and 6 months time point, respectively (p=0.0001). However, from 6 to 18 months time points, there was only marginal reduction in mf loads from 1.33 (21.0%) to 1.11 (17.6%) with no significant difference (p=0.133) at both 6 and 18 months. Volunteers who were in the ivermectin and albendazole semi-annual treatment arms recorded significant reductions in mf geometric loads from 5.36 (100%) to 1.22 (22.7%) at pretreatment and six months time point, respectively (p=0.0001), representing and also a marginal but no significant reductions in mf loads from 1.22 (22.8%) to 1.08 (20.2%) at 6 and 18 months time point, respectively (p=0.061).

Table 10 Assessment of microfilaria loads of volunteers at pretreatment, 6 and 18 months time points

Treatment arms	**Pre-treatment Geometric mean (Gm.) of mf/mg**	**6-months Geometric mean (Gm.) of mf/mg**	**P-value**
IVM alone annually	(100%)	1.26 (20.59%)	P<0.0001◆
	6-months Gm. of mf/mg	**18-months Gm. of mf/mg**	**P-value**
	1.26 (20.59%)	1.10 (17.97%)	P=0.29◆
IVM alone semiannually	**Pre-treatment Gm. of mf/mg**	**6-months Gm. of mf/mg**	**P-value**
	5.27 (100%)	1.25 (23.72%)	P<0.0001 ◆
	6-months Gm. of mf/mg	**18-months Gm. of mf/mg**	**P-value**
	1.25 (23.72%)	1.02 (19.35%)	P=0.002 ◆
IVM plus ALB annually	**Pre-treatment Gm. of mf/mg**	**6-months Gm. of mf/mg**	P-value
	6.32 (100%)	1.33(21.04%)	P<0.0001◆
	6-months Gm. of mf/mg	**18-months Gm. of mf/mg**	**P-value**

	1.33(21.04%)	1.11 (17.56%)	P=0.133◆
Treatment arm	**Pre-treatment Gm. of mf/mg**	**6-months Gm. of mf/mg**	**P-value**
IVM plus ALB semiannually	5.36 (100%)	1.22 (22.76%)	P<0.0001 ◆
	6-months Gm. of mf/mg	**18-months Gm. of mf/mg**	**P-value**
	1.22 (22.76%)	1.08 (20.15%)	P=0.061◆

◆-Wilcoxon Signed Rank Test

CHAPTER 5 - DISCUSSION

5.0 INTRODUCTION

Currently, ivermectin is the sole drug approved by the World Health Organization (WHO) for use in onchocerciasis control programs (Awadzi *et al.*, 2003). Albendazole is also known to be effective against all intrauterine stages of *O. volvulus* and can be taken safely in combination with ivermectin (Awadzi *et al.*, 1995b; Makunde *et al.*, 2003). Demonstrations of *O. volvulus* responses to antifilarial chemotherapy treatment can unambiguously be determined by sequential determination of skin snip microfilariae counts before and after treatment (Awadzi, *et al*., 2004a). In this study, microfilariae levels were monitored at pre-treatment, six and 18 months time points to assess the efficacy of ivermectin alone and ivermectin plus albendazole against *O. volvulus* administered annually and biannually among some volunteers in Adansi South District.

5.1 LEVEL OF ENDEMICITY OF *O. VOLVULUS* INFECTION

In this study, the prevalence of onchocercal nodules and microfilariae were used to determine the level of endemicity of *O. volvulus* infection in the study communities,

following the criteria of the Onchocerciasis Elimination Programme for the Americas (OEPA) where nodule and microfilarial prevalence of ≤ 20%, 21-59% and ≥ 60% are defined as hypoendemic, mesondermic and hyperendemic respectively. Out of 2326 volunteers examined for onchocercal nodules, 42.2% were found to be nodule positive. Therefore, nodule prevalence assessment alone indicates that the Adansi South District is meso-endemic for *O. volvulus*. In the study population, 43.5% and 41.1% out of the total male and female volunteers examined respectively had palpable nodules indicating that the prevalence of nodules were higher in male than in female volunteers. Sex related nodule prevalence has been reported in other studies and it has been attributed to the differences in degree and frequency of duration of how males and females participants are exposed to the bite of infective *Simulium* vectors, with males participants being more exposed (Anosike *et al.*, 2001; Opara and Fagbemi, 2008).

In this study, a total of 37.5% microfilariae prevalence was obtained among study volunteers who had at least one palpable nodule in Adansi South district. Microfilariae assessment also confirms that the prevalence of *O. volvulus* infection is higher in males than in females with prevalence being 41.4% and 31.6% for males and females respectively.

According to unpublished data by Ghana Health Service in 2009, the district is hyper-endemic for *O. volvulus* infections. However, nodule prevalence in this study categorized the district as meso-endemic for *O. volvulus* infections. The change in the endemiciy of *O. volvulus* levels from hyper-endemic to mesoendemic district could be attributed to the impact of ivermectin mass drug administration (MDA) (Opara and Fagbemi, 2008) going on in the district and the activities of unlawful mining in and along both River Pra and River Offin for the past 5 years in the district.

These mining activities could disturb the natural niche of the *Simulium* vectors (Cheke *et al.*, 2015). This reduction in endemicity could also be attributed to the fact that ivermectin mass drug administration has been ongoing for the past 10 years in this district.

5.2 EFFECT OF STUDY DRUGS ON MICROFILARIDERMIA STATUS OF STUDY VOLUNTEERS

Assessment of the microfilaridermic volunteers 6-months post-treatment in this study revealed that, there were significant and consistent reductions in the number of microfilaridermic volunteers in all the four treatment arms. These observations could be attributed to the microfilaricidal and embryostatic effect of ivermectin with no added effect from albendazole. Ivermectin is known to have a microfilaricidal effect with efficacy of 99% and embryostatic effect (Basáñez *et al.*, 2008b), which leads to cessation of microfilariae production immediately after treatment. However, recovery of microfilariae production by adult *O. volvulus* is known to gradually start from 3 to 6 months post treatment with ivermectin (Plaisier *et al.*, 1995), even though some adult female worms do not resume microfilariae production for about 18 months after ivermectin treatment (Bottomley *et al.*, 2008). Albendazole has no microfilaricdal effect but rather it affects intra-uterine stages of *O. volvulus* (Awadzi *et al.*, 1995a). These aforementioned activities of ivermectin alone could explain the significant reductions in the number of volunteers who were microfilariae-positive at 6 months follow up in all the treatment arms.

The results from this study also indicated reductions in microfilaridermic volunteers from 6 months to 18 months treatment time point in all the treatment arms. The analysis of results from this present study also showed significant difference in the number of microfilariae volunteers treated with ivermectin alone annually and ivermectin alone bi-annually and no significant difference was seen in the number of microfilaridermic volunteers treated with ivermectin plus albendazole annually and ivermectin plus albendazole bi-annually. However, volunteers who received either ivermectin alone or ivermectin plus albendazole biannually recorded low number of microfilardermic volunteers compared to those treated on the annually bases. These observations could be attributed to the fact that ivermectin has microfilaricidal and embryostatic effect which temporally ceases microfilariae productions by adult female worms. The embryostatic effect of ivermectin causes the production of microfilariae to reach a new production level, which is reduced irreversibly by an average of 30% to 35% after each ivermectin treatment round (Plaisier *et al.,* 1995). Therefore each round of ivermectin exerts a cumulative reduction effect on female adult worm fertility (Winnen *et al.,* 2002). This cumulative reduction effect of ivermectin which arises as a result of embryostatic activities of ivermectin (Plaisier *et al.,* 1995) could explain the lower microfilaridermic volunteers recorded in the bi-annual treatments groups compared to the annual treatment arms.

5.3 EFFECT OF STUDY DRUGS ON MICROFILARIA LOADS

The results indicated drastic reductions in microfilariae loads from pre-treatment to six months follow-ups in all the treatment arms. This was followed by marginal mf loads reductions from 6 months to 18 months time points in all the treatment arms. However, volunteers treated with ivermectin alone bi-annually and ivermecin plus

albendazole semi-annually recorded the highest reductions in microfilariae loads at 18 months follow-up. The reductions in microfilariae loads at 6 and 18 months follow-ups in all the treatment arms could be attributed to the potent microfilaricidal and embryostatic activity of ivermectin with no added effect from albendazole (Awadzi *et al.*, 2003). However the lower microfilariae loads among bi-annual treatment arms compared to its annual counterparts at 18 months time point could be attributed to embryostatic effect of ivermectin on adult female which temporally blocks the release of microfilariae (Basáñez *et al.*, 2008a), exerting a reduction cumulative effect on microfilariae productions at each round of ivermectin treatment (Turner *et al.*, 2013). This means that the rate of microfilariae productions are progressively reduced with each treatment round of ivermectin (Winnen *et al.*, 2002).

Also, at 18 months follow-up, result from this study showed lower geometric mean microfilariae loads in the ivermectin alone annual treatment arm than the combination therapy of ivermectin and albendazole annual treatment arm. This could be due to the fact that albendazole may be reducing the potency of ivermectin. Dreyer and others in 2006, also observed that albendazole reduces the macrofilaricidal activity of diethycarbamazine (DEC) which is an approved microfilaricidal drug for the treatment of filariasis (Dreyer *et al*., 2006).

Comparative assessment of skin microfilariae loads revealed no significant differences among all the four treatment arms at each time point. Awadzi and colleagues reported a similar trend in microfilaridermic volunteers treated with ivermectin alone and ivermectin plus albendazole. They observed that combined treatment of 150µg ivermectin plus 800mg of albendazole produced similar efficacy in the suppression of skin microfilariae when compared to ivermectin alone (Awadzi

et al., 1995a). This means that, the observed reductions in the geometric mean microfilariae at 6 and 18 month-time points in all the treatment arm in this present study could be attributed to the microfilaricidal effect of ivermectin with no added effect from albendazole.

5.4 ADVERSE EVENTS ASSOCIATED WITH STUDY DRUGS

A serious adverse event has been defined by the international conference on harmonization of technical requirements for registration of pharmaceuticals for human use (ICH), as " any untoward medical occurrence that at any dose results in death, is life threatening, requires inpatients hospitalization or prolongation of an existing hospitalization, resulting in persistent or significant disability/incapacity and/or is a congenital anomaly or birth defect" (WHO, 2001). These criteria have been modified by WHO to include important medical events that may in fact put the patient in danger or will need intervention to prevent death of a patient (Awadzi *et al.*, 2003).

No serious adverse event was reported as a result of drug administration in this study. This observation confirms the findings from adverse reactions assessment after a large-scale treatment of onchocerciasis with ivermectin, which suggested that, ivermectin causes minimal side effects and is sufficiently free of severe reactions (De Sole *et al.*, 1989). Some of the adverse events reported in this study included swelling of limbs, swollen face, ocular reactions, cutaneous reaction (itching), cutaneous reaction (rash), nausea, and dizziness which are already known to be associated with ivermectin. However, cutaneous reaction was the most reported

adverse event at all the treatment time points and this could be explained by the fact that adverse reactions which occur as a result of ivermectin treatment are triggered by dying microfilariae which predominantly live in the skin of infected people (Hopkins and Boatin, 2011). When microfilariae die, they produce inflammatory reactions, which may be small and localized but due to the large number of microfilariae dying as result of ivermectin treatment within a short period, these inflammatory reactions are wide spread (Hopkins and Boatin, 2011).

Studies, however, have shown that *Wolbachia* endobacteria within *O. volvulus* may contribute to the inflammatory reactions associated with onchocerciasis (Debrah *et al.*, 2007; Taylor *et al.*, 2010).

In this study, treatment start time point recorded the highest number of volunteers who reported adverse events whiles 12 months follow up recorded the least number of volunteers who reported adverse events. This indicates that, the number of volunteers who reported adverse events decreased with increasing treatment. This observation could be attributed to the fact that the occurrence of adverse reactions after ivermectin treatment is directly related to the death of worms (Cross *et al.*, 2001). This observation thus confirms findings by Ottessen and colleagus who reported that the occurrence of adverse reactions after anti-filarial treatments is proportional to pre-treatment microfilariae levels (Ottesen *et al.*, 1999). Furthermore, at 6 months treatment time point the annual treatment arms recorded the lowest number of volunteers who reported adverse reactions compared to volunteers in the semi-annual treatment arm. This observation could be due to the fact that volunteers in the annual treatment arms received vitamin C alone while volunteers in the semi-annual treatment arms received either ivermectin alone or ivermectin plus albendazole. Meanwhile at 12 months the semi-annual treatment

arms recorded the lowest number of volunteers with adverse reactions compared to the annual treatment arms. This observation could be attributed to the fact that, volunteers in the semi-annual treatment arms received ivermectin at 6 months, which could result in low number of microfilaridermic volunteers among them at 12 months. This supposed difference in the number of microfilaridermic volunteers between annual and semi-annual treatment arms at 12 months could explain the differences in the number of volunteers who had adverse events at 12 months treatment time point.

CHAPTER 6 – CONCLUSION AND RECOMMENDATIONS

6.0 CONCLUSION

Ivermectin, the current drug of choice for the control and treatment of onchocerciasis is still effective in clearing skin microfilariae of volunteers in the Adansi South District of Ghana where mass drug administration (MDA) of ivermectin has been ongoing for the past 10 years.

Biannual treatment of ivermectin alone and ivermectin plus albendazole were found to have additional benefit in reducing microfilariae prevalence compared to annual treatment.

This study also suggests that, co-administration of ivermectin (200µg) and albendazole (800mg) is as effective as ivermectin (200µg) alone in clearing skin microfilaria of individuals infected with *O. volvulus.*

6.1 RECOMMENDATIONS

It is recommended that:

i. Mass drug administration of ivermectin against *O. volvulus* infections should be organized semi-annually in communities where transmission of microfilariae is still ongoing. This will stop mf from coming to the skin and hence stop transmission.
ii. Further investigations should also be done to assess the macrofilaricidal efficacy of ivermectin (200µg/kg) plus albendazole (800mg), which could be seen at a longer time point, that is within this study.

iii. Studies should also be done to assess the impact of unlawful mining activities in and along River Offin and Pra on *Simulium* vector infective rate in the district.

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