Addis Ababa University

Graduate Studies Program

Biology Department

Identification of the Etiology of *Elephantiasis* in Midakegn District, Western Shoa Zone, Oromiya Region

By *GELETA GESHERE OLI*

A Thesis Submitted to the Graduate Studies Program of Addis Ababa University in Partial Fulfillment of the Requirement for the Attainment of the Degree of Master of Science in Biology
(*Biomedical Science)*

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List of Abbreviations

χ^2	Chi square test
^0C	Degree centigrade
ICT	Immunochromatographic test
Kms	Kilo meters
SNNP	South Nations and Nationalities of People
Zr	Zirconium
Be	Beryllium
Fe	Iron
Al	Aluminium
Si	Silicon
CFA	Circulating Filarial Antigen
DEC	Diethylcarbamizene
LPS	Lipopolysaccharide
IFN-γ	Interferon gamma
IgG	Immunoglobulin G
TNF-α	Tumor necrosis factor alpha
NO	Nitrogen Oxide
IL	Interleukin
L_1, L_2, & L_3	Larval stages 1, 2, & 3
ADLA	Acute dermatolymphangioadenites
AFL	Acute filarial lymphangitis
AM	Antemeridian
PM	postmeridian
TDR	Tropical disease rank
DALYs	Daily adjusted life years
CDC	Communicable disease control
a.s.l.	Above sea level
WHO	World Health Organization
Mf	microfilariae

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Abstract

Elephantiasis is mainly caused by blockage of lymphatic channels by lymphatic filarial parasites or by soil borne minerals (Silica, Aluminum, etc.) derived from volcanic rocks that enter through the skin of the barefooted persons who work on farms. Endemic non-filarial elephantiasis is a major public health problem in countries of tropical Africa, Central America, and North India. The present study attempted to determine the etiology of elephantiasis in two kebeles (Ganbella and Tullu Eteya) of the Midakegni district, West Shoa Zone, Oromiya Region. The method used included physical and clinical examinations, blood film examination, serological diagnosis, and interviews. Physical observations were made on 1656 residents of age >14, 914 were from Ganbella and 742 from Tullu Eteya kebeles. From this population, 123 persons were positive for elephantiasis of the lower legs, of which 67(4.04%) males and 56 (3.38%) females (age range 14 to 70 years). Therefore, based on the local population census the prevalence of elephantiasis in the area was estimated to be 7.42 %. From the elephantiasis positive study participants about 49 % were also selected randomly for further serological diagnosis. From which the highest prevalence (78%) is among the most economically productive age group (21 – 60 years old). However, clinical examination of participants with elephantiasis showed no hydrocele or scrotal swelling, which is suggestive of a lymphatic filarial etiology. Furthermore, the parasitological diagnosis of blood collected from patients with elephantiasis between 10 A.M. and 2 A.M. and serological diagnosis by using immunochromatographic test (ICT) kit for filarial antigens in the blood samples were all negative. This makes it unlikely that the etiology of elephantiasis in the present study area was lymphatic filarial parasites. On the other hand, the house-to-house survey revealed a strong association between being bare footed and having elephantiod swelling of the feet and legs. This association, together with the red clay soil of the area, which is typically associated with podoconoisis in other parts of the country and globally, suggests the etiology of the elephantiasis to be soil derived mineral particles in the present study area. The study has also shown the importance of footware in the prevention of non-filarial elephantiasis. Further study will be required to assess the economic impact of the disease in the Midakegni district, as the disease is mainly affecting economically productive age groups in the area.

Key words: Elephantiasis, Etiology, Immunochromatographic test, House-to-house survey, Podoconiosis

1. Introduction

Elephantiasis is chronic, often extreme enlargement and hardening of cutaneous and subcutaneous tissue, especially of the extremities, breast, and external genitalias resulting from lymphatic obstruction and usually caused by infestation of the lymph glands and vessels with a filarial worm or by absorption of ultrafine silicates of red clay soil. It may be a chronic filarial disease, usually seen in the tropics, due to infection with lymphatic filarial parasites, and marked by inflammation and obstruction of the lymphatic, and hypertrophy and thickening of the skin and the surrounding tissues, and subcutaneous tissues, chiefly affecting the legs and external genitals of humans, or hypertrophy and thickening of the tissues from any cause.

Elephantiasis was noted (600B.C.) by ancient Hindu and Persian physicians who were the first to forward that the disease was probably due to *Wuchereria bancrofti*. Because of this elephantiasis is considered as one of the oldest and most debilitating diseases in the world. For instance, centuries-old art and texts from Egypt, Japan, Africa, Persia and India depict the disease (Jones et al., 1972). Further more, recently, it has been understood that either filarial parasites or non-filarial agents of geologic origin can cause elephantiasis, which is the gross swelling of the soft tissues mostly of leg, arm, breast, and external genital organs, or only lower legs, respectively that results from obstruction of the lymphatic flow by either of the two agents.

Elephantiasis can be caused mainly by lymphatic filariasis (by three different species of filarial worms such as *Brugia malayi*, *Brugia timori* and *W. bancrofti*) and non-filarial elephantiasis, and sometimes by onchocerciasis, lepromatous leprosy, surgical resection or trauma, lymph node destruction by tuberculosis, chromomycosis, metastasis, genital infection with certain strains of *Chlamydia trachomatis* (lymphogranuloma venereum), and severe urinary schistosomiasis (Gupta et al., 2006). However our focus is on elephantiasis that caused by the parasites and the soil minerals.

Elephantiasis that results from repeated infections by filarial parasites (*W. bancrofti, B. malayi and B. timori)* over longer periods of time is the products of a complex

immunogenic reaction (Bush et al., 2001), and partly from the recently implicated endosymbiont *Wolbachia* of the filarial parasites (Taylor et al., 2001). Lymphatic filariasis is one of the leading causes of permanent and long-term disability in the world, after leprosy. The disease transmits from person-to-person by mosquito vectors in which the filarial life cycle that involves the uptake of the microfilariae by mosquitoes, development to third stage larvae and transmission back to the vertebrate host takes place.

In Africa, where filariasis is endemic *W. bancrofti* is the only etiologic agent of elephantiasis (WHO, 1984), whereas, where filariasis is not endemic the physicochemical etiology (absorption of silicate particles through the skin abrasion) is common (WHO, 1987).

This disease principally affects people in tropical and sub-tropical areas of Asia, Africa and Latin America. WHO estimates that a billion people are at risk in about 80 countries and over 120 million have already been affected by the disease, and over 40 million of these are seriously incapacitated and disfigured by the disease. One third of the people infected with the disease live in India, one third are in Africa and the rest are in South Asia, the Western Pacific, and parts of Central and South America (deVries, 2005).

Jones et al., (1972) and Babu et al., (2005) have shown that approximately one third of infected individuals present with overt clinical manifestations lymphoedema and elephantiasis of the limbs or genitals, hydrocoele, chyluria or recurrent infections associated with damaged lymphatic; the remainder is at the pre-clinical stage of infection (most often with microfilaria in their blood). In addition to this burden, this disease has serious psychosocial consequences. Because of these great impacts lymphatic filariasis has been identified by WHO as one of the six diseases, which could be targeted for elimination/eradication based on considerations that human beings are the only reservoir infection.

``Non-filarial elephantiasis is a chronic disorder characterized by the very slow onset of oedema, subsequent lymphoedema and later elephantiasis that mostly limited to below the knee. The disease is caused by immunological response to certain minerals (silicates, zirconium or beryllium-containing mineral soils). When walking barefoot on ground containing these minerals, the soil particles can be absorbed through the soles of the feet via small wounds. They are then transported via the lymphatic to the inguinal lymph nodes where they cause a local inflammatory reaction. Atrophy and fibrosis of the lymphatic occur subsequently in contrast to lymphatic filariasis where dilatation occurs. The disorder occurs in well-defined areas (specific mineral composition of the soil) in people who walk barefoot, such as Ethiopia, Kenya, Rwanda, Uganda, West Africa and India. Whereas lymphatic filariasis occur predominantly in lowland areas (vector biotope), non-filarial elephantiasis is characteristically of highland zones. This, however, is not absolute. Other signs of lymphatic filariasis such as hydrocele, eosinophilia, and nocturnal microfilaraemia are absent in this disease. ``
(http://www.cigna.com/healthinfo/nord689.html, 24/04/2007)

1.1. Filarial elephantiasis

Demarquay discovered microfilariae in hydrocele fluid in 1863. By 1900, the whole life cycle of *W. bancrofti* was elucidated; it was Patrick Manson's discoveries that showed for the first time that arthropods were a vector for the parasitic disease. Currently, it has been established that mosquitoes are the vectors of this parasite including *Brugia malayi* and *Brugia timori*.

Filarial elephantiasis results from infection with the nematode parasites (*W.bancraft:, B. malayi and /or B. timori).* The gross enlargement of lower extremities due to lymphatic infection by the filarial parasites was recorded in the ancient medical literature of china, Egypt, India, Japan and Persia and was referred to as elephantiasis arabicum by western physician since antiquity (Sasa, 1976; Grovz, 1990; and Nelson , 1996) cited by Muller and Wakelin (1998).

Among the eight tissue-dwelling vector-borne filarial nematode species, *W. bancrofti, B. malayi* and *B. timori* naturally cause elephantiasis in humans. *W. bancrofti* is the most

widely distributed of the three species of lymphatic dwelling filariae. The infection damages the lymphatic system of the lower extremes, breast, scrotum and/or arms of human. The pathology of the three filarial species is primarily associated with acute and chronic changes in lymphatic system resulting in elephantiasis, the gross enlargement of limbs and hydrocele, the scrotal sac swelling. The disease is thought to be caused primarily by adult worms living in the lymphatic vessels. The microfilariae released by the female worms circulating in the peripheral blood are not harmful (Sherchand et al., 2003). This might be attributed to the fact that parasitaemic hosts develop a parasite stage specific immunological tolerance and fail to mount an antibody response to the microfilarial sheath surface proteins (Jones et al., 1972 and Babu et al., 2005) because the microfilariae are completely surrounded by a bag-like, antibodies-impermeable structure, the microfilarial sheath, which derives from the embryonic eggshell, but, is altered by maternal components secreted by the uterus epithelium (Miranda et al., 2005). Lymphatic filariasis is also one of the most prevalent tropical diseases. The disease is reported to be responsible for 5 million daily adjusted life years (DALYs) peoples, annually ranking 3rd among the TDR diseases in terms of DALYs, after malaria and Tuberculosis and is major impediment to socioeconomic development and is responsible for immense psychosocial suffering among the affected (www.who.int/tdr, 12/25/2005).

Lymphatic filariasis caused by *W. bancrofti, B. malayi* and *B. timori*, transmitted by a number of mosquito species, is a major cause of morbidity, affecting all ages and both sexes. The prevalence of lymphatic filariasis is increasing in many areas of the tropics, where vector habitat has expanded because of large-scale water projects and declining sanitation associated with uncontrolled urban growth (Albuquerque et al., 1995) even though WHO targeted and trying to eliminate and/or eradicate by 2020. Consequently, recently, more than a billion people (around 18% of world's population), who are living in areas endemic for lymphatic filariasis in more than 80 countries mainly in Africa, Asia and to a lesser extent in Latin America are at risk of infection and over 120 million cases of lymphatic filariasis either having patent microfilaraemia or chronic filarial disease (Michael et al., 1996), over 40 million of them are seriously disabled and disfigured by the disease in the world of which two-third of the people infected with the disease live in

India and in Africa, and most of the remainder are in south Asia, the Pacific and Americas (http://www.who.int/mediacentre/factsheets/fs102/en/, 3/7/2006). Ninety five percent of these infections are caused by *W. bancrofti*, and the remainder percents of infections are by *B. malayi* and/or *B. timori* (www.who.int/tdr, 12/25/2005).

The burden of the disease and its socioeconomic consequences are concentrated in tropical and subtropics areas (Leang et al., 2004). Although death because of elephantiasis is rare, clinical suffering and disability are very common among the infected people. It is expressed in ICMR Bulletin (2002) that filariasis is a disease of poor and is a cause and effect of poverty. The majority of the people at risk of filariasis live in rural areas. Poor sanitations together with low socioeconomic status of the human population make the environment conducive for proliferate breeding of vector mosquitoes facilitating transmission (ICMR Bulletin, 2002). As a result lymphatic filariasis is considered as an emerging disease in many areas of the tropics, where vector habitat has expanded because of large-scale water projects and declining sanitation associated with uncontrolled urban growth (http://www.cdc.gov/ncidsd//, 09/25/2006).

1.1.1. Lymphatic filarial worms

The lymphatic filarial parasites share a number of morphological and life cycle characteristics, in that they are long slender worms living in lymphatic niches, with prolonged developmental cycles and dependent on vector transmission. Adult worms are long and slender with a smooth cuticle; bluntly rounded ends; and pale, threadlike structure (deVries, 2005). The head is slightly swollen and bears two circles of well-defined papillae. The mouth is small and is without buccal cavity. The vulva is near the level of the middle of the esophagus of female.

W. bancrofti displays a large size gap between the male and female. The adult male worm is long and slender, between four and five centimeters in length, a tenth of a centimeter in diameter, and features a curved tail. The adult female, in contrast, is measuring 6-10 cm in length and three times larger in diameter than the male. This size deviation can be

attributed to the vast numbers of microfilariae that the female produces each day (WHO, 1987).

1.1.2. Life Cycle of lymphatic filarial worms

Among the agents of lymphatic filariasis, *W. bancrofti* is dominant in tropical areas worldwide; *B. malayi* is limited to Asia; and *B. timori* is restricted to few islands of Indonesia (http://www.Dpd.cdc.gov/DPDX/HTML/filariasis.htm/, 07/15/2006). The adult lymphatic-dwelling parasites survive for years and the viviparous females release large amounts of first stage larvae, microfilariae, into the blood stream.

W. bancrofti carries out its life cycle in two hosts; human beings that serve as the definitive host and mosquitoes as its intermediate hosts (Fig. 1). For *W. bancrofti*, humans are the exclusive host, and even though certain strains of *B. malayi* can also infect some felines and monkeys, the life-cycles in humans and in these other animals generally remain epidemiologically distinct so that little overlap exists (deVries, 2005).

The adult female parasite that resides in the lymphatic system is viviparous and produces millions of microfilariae that are released in to the lymph and find their way into blood circulation via the thoracic duct. Microfilariae, the first stage larvae are present in the circulation and migrate between the deep and the peripheral circulation.

Female mosquitoes bite infected humans and pick up *W. bancrofti* microfilariae that are circulating in the blood. The ingested microfilariae shed their sheaths; penetrates the stomach wall; migrate to the muscles of the thorax; and develop there without multiplication unlike plasmodium. The slender active microfilaria transforms to the short thick inactive sausage – stage larva (L_1) that characterized with conspicuous slender tail which is formed from cuticle while in the genus *Brugia* one or two nuclei are present inside the tail (WHO, 1987). After the first molt, the larva grows rapidly in length and width, and become potentially more active, although usually it does not move. This pre-active larva (L_2) is recognized by its short tail. After a 2^{nd} molt the parasite no longer has

visible cuticle becoming infective (L_3) larva. This larva grows further in length but not in width, moving actively in the haemocoelic cavity of the mosquito, first towards the abdomen and later to the head and proboscis from which get entry to human upon the second blood-meal of the mosquitoes (http://www.who.int/mediacentre/factsheets/fs102/en/, 05/20/2006).

When their current host feed, and the microfilariae are egested into the blood stream of its new human host. The larvae move to the lymph nodes, predominantly in the legs and genital area, and develop into adult worm over the course of a year. By this time, an adult female can produce microfilariae.

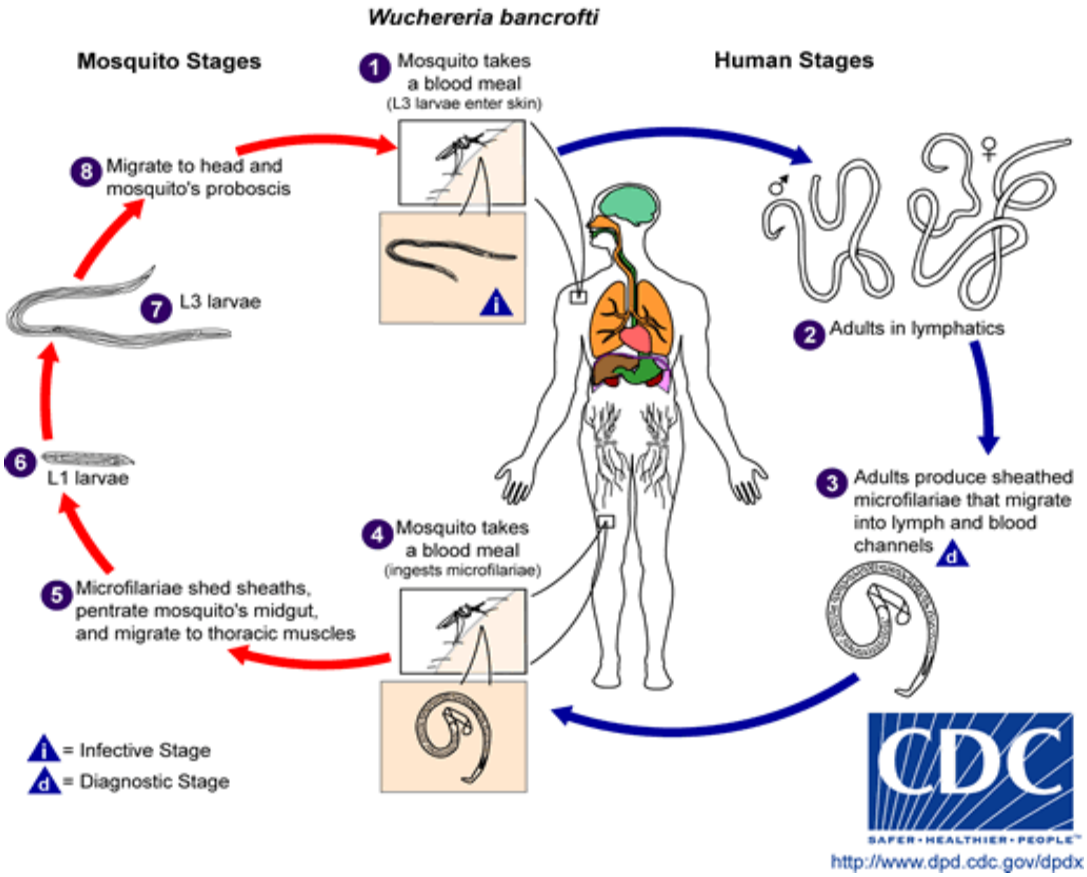

Fig. 1. Life cycle of *Wuchereria bancrofti*

Source: (http://www.dpd.cdc.gov/dpdx/HTML/Filariasis.htm, 20/042007)

The juvenile and adult worms normally live in the lymph vessels and the microfilariae are also found in the blood circulation. The adult parasites can live for many years (probably up to 10 years) whereas the lifespan of the microfilaria is about a year at the most although the duration of larval development is directly related to temperature, that is, the warmer temperature the more rapid development. It usually takes 10 – 14 days for *W. bancrofti* to reach the infective stage, and 7-10 days for *B. malayi* and *B. timori* (WHO, 1987).

1.1.3. Periodicity of microfilariae

In humans, the microfilariae show a characteristic periodicity in the course of the circadian or 24 hour cycle. In most regions of the world where filariasis is endemic, *W. bancrofti* microfilariae are of periodic type (Muller and Wakelin, 1998). They live mainly in the pulmonary capillaries from where a proportion of them escape into the peripheral blood where they may be detected during the hours of their periodicity (WHO, 1987). The nocturnally periodic parasites show a marked peak of microfilariae density in the peripheral blood during the night hours, mostly between 10 p.m. and 4 a.m. (Garcia and Bruckner, 1997).

Rural *W. bancrofti* is nocturnally periodic over most of its ranges. It is mainly transmitted by several species of *Anopheles* that breed in a wide variety of water body and those mostly rest indoors, and bite at night either indoors or outdoors (Service, 2000). During the day the microfilariae are present in the deep veins and during the night they migrate to the peripheral circulation where they can be available for the vectors. This indicates that the prediocity of microfilariae of *W. bancrofti* coincides with the biting activities of the local vector (WHO. 1987).

Gatika et al. (1994) has confirmed that the density of microfilariae of *W. bancrofti* become peak at 4min. to 1 a.m. by counting microfilariae from stained blood film collected within 24hr cycle at 2hr interval from the infected persons in Kenya. This implies that the parasite is periodically nocturnal in the eastern Africa. Similarly, Jemaneh and Kebede (1995) have shown that the majority of microfilariae appear

between 8 p.m. and 4 a.m. depicting the nocturnal periodicity of circulating *W. Bancroft* microfilariae at Gambella, southern Ethiopia

1.1.4. Vectors of lymphatic filarial worms.

The major vectors for *W. bancrofti* are *Culex* mosquitoes in most urban and semi-urban areas, *Anopheles* in the more rural areas of Africa and elsewhere, and Aedes species in many of the endemic Pacific islands. For the *Brugian* parasites, Mansonia species serve as the major vector, but in some areas *Anopheline* and *Aedes* mosquitoes are responsible for transmitting the infection. *Brugian* parasites are confined to areas of eastern and southern Asia especially India, Malaysia, Indonesia, the Philippines and China **(**deVries, 2005**).**

Specifically, *Culex quinquefasciatus*, *Anopheles gambiae, Anopheles funestus*, and *Aedes polynesiensis* are the most common species of mosquitoes that transmit *W. bancrofti* in the world (WHO, 1987). Moreover, it has been established, in tropical Africa, *Culex* and *Anopheles* species are the main vectors for the periodic *W. bancrofti* and the vector, *Culex quinquefasciatus* is found only in African continent, especially along the coast of the Indian Ocean, (of Kenya and Tanzania) (WHO 1987). In Ethiopia, only *Anopheles* species (*A. gambiae & A. funestus*), were identified during a limited surrey made for *W. bancrofti* in Gambella region, south western Ethiopia (McConnell & Schmidt, 1973).

1.1.5. Clinical manifestations

Elephantiasis is the second leading cause of long-term disability to mankind world wide and is characterized by acute (adenolymphangitis, fever, and inflammatory episodes) and chronic (hydrocele, chyluria, lymphodema, and elephantiasis) manifestations of the infection with lymphatic-dwelling parasites mainly the species *W. bancrofti* (Behm et al., 2005); and by hyperplasic and fibrosis of the subcutaneous tissue and thickening of the skin (WHO, 1987). The disease manifests as progressive lymphodema leading to disfiguring elephantiasis in both genders (ICMR Bulletin, 2002). In addition, as the adult worms live in the vessels of the lymphatic system, especially of the legs, arms, scrotum and breasts, they cause the vessels to dilate so that the lymph fluid moves slowly and

ineffectively. On other hands, tropical pulmonary eosinophilia is a potentially serious progressive lung disease with nocturnal cough, sneezing and fever, resulting from immune hyper-responsiveness to microfilariae in the pulmonary capillaries (http://www.cdc.gov/travel/diseases/filariasis.htm, 20/07/2006).

The onset of symptoms is slow, but the effects are very apparent after several years. During the early inflammatory stage, a host can exhibit intermittent fever; enlarged, tender lymph nodes mostly of the inguinal lymph nodes, granulation lesions, and impaired circulation (http://www.gsbs.utmb.edu/microbook, 06/03/2006). Following, the lymph nodes are well enlarged and dilated, and become hardened and clogged with fibrous tissue that prevents the lymphatic system from operating correctly. Without the proper drainage of fluids, the affected tissue will expand resulting in elephantiasis, a gross expansion of body; followed sometimes by death.

Filariasis results in hidden infection; as most asymptomatic carriers have sub-clinical lymphatic damage and hydrocele, the predominant manifestation of bancroftian filariasis which is also not revealed normally, unless it results in gross scrotal enlargement (Pani et al., 1991). In addition, skin changes such as skin fold thickening, hyperkeratosis, pigmentary changes, chronic ulceration, epidermal and sub-epidermal nodules were observed and compared between the different lymphoedema grades (Burri et al., 1996). These lesions are not specific to chronic lymphatic filariasis, and have been described in other conditions displaying lymphostasis and are thought to be favored by secondary infections. In filarial lymphodema, there is progressive persistent inflammation (chronic dermatitis) due to the invasion of bacteria esp. beta haemolytic streptococci in the skin (Pani et al., 1991).Infections may be asymptomatic or have a wide range of signs and symptoms primarily attributed to inflammatory reactions and obstructive changes in the lymphatics. Hypersensitivity also has a major role in pathogenesis. Pani and Srividya (1995) have suggested that the progression of lymphoedema from one grade to the next in bancroftian filariasis is associated with increased frequency of adenolymphangitis attacks that results from secondary bacterial infections.

The natural history of lymphatic disease in human filariasis remains unclear, but recurrent episodes of acute lymphangitis are believed to constitute a major risk factor for the development of chronic lymphoedema and elephantiasis. However, prospective analysis study made in clinic indicated that two distinct acute syndromes accompanied by lymphangitis occur in patients of filariasis-endemic areas. One syndrome is acute filarial lymphangitis (AFL) that caused by the death of adult worms, and which is relatively uncommon in untreated persons, usually is asymptomatic or has a mild clinical course, and rarely causes residual lymphoedema. The second syndrome, of acute dermatolymphangioadenitis (ADLA), is not caused by filarial worms as such, but probably results from secondary bacterial infections; and is a common cause of chronic lymphoedema and elephantiasis even where lymphatic filariasis is not present (Dreyer et al., 1999). Similarly, recurrent attacks of episodic adenolymphangitis result in progression of chronic lymphodema from early reversible to late irreversible elephantiasis (Pani et al., 1991). Further more, although the factors responsible for the initiation and progression of filarial lymphodema to its most severe form, elephantiasis, have been debated, recurrent episodes of bacterial acute dermatolymphangioadenitis (ADLA) play a major role (Dreyer et al., 1999 and Tisch et al., 2005). ADLA is characterized by painful swelling of the limb and is accompanied by fever and chills lasting for several days, sometimes with nausea and vomiting (Ramaiah et al., 1996 and Dreyer et al., 1999). As lymphodema progresses, the frequency of ADLA episodes generally increases (Pani et al., 1995). Skin changes of chronic lymphoedema include thickening, nodular lesions, and pigmentary changes (Burri et al., 1996 and Olszewski et al., 1993).

Hydrocele, even though found only with *W. bancrofti* infections (that means not with Brugian infections), is the most common clinical manifestation of lymphatic filariasis (deVries, 2005) and is one of the chronic consequences of bancroftian filariasis in 40-50% of men living in the highly endemic areas (Kumarasswami, 2000). Similarly, in any endemic areas 10 – 50% of men suffer from genital damage; especially, hydrocele (the fluid-filled balloon-like enlargement of the sacs around the testes) and elephantiasis of penis and scrotum. This is the worst symptoms of the chronic disease generally appearing

more in adult men than in women (http://www.who.int/mediacentre/factsheets/fs102/en/, 20/05/2006). Genital elephantiasis can also be caused by bacterial infection those sexually transmitted diseases, specifically lymphogranuloma venereum. The bacterium that results in lymphogranuloma venereum, Chlamydia trachomatis serovar L_1-L_3, damages the lymphatic system resulting in lymphatic obstruction in the genitals which in turn eventually results in genital elephantiasis (Gupta et al., 2006).

Chyluria is another form of the chronic filarial syndromes, is caused by the intermittent discharge of intestinal lymph (chyle) in to the renal pelvis and subsequently in to the urine (deVries, 2005).

In areas where bancroftian filariasis is endemic, the clinical manifestations of the disease, which are often very varied, appear most frequently during early adulthood or later, but clinical and pathological observations made by Figueredo and Dreyer (2005), in Brazil, on 22 children (aged 2-15 years) who were infected with *W. bancrofti*, has showed that the adult worms were predominantly located in the lymph-node and in the afferent or efferent vessels of draining lymph nodes, mainly in the inguinal region.. They also observed histologically that the change in the lymphatic vessels and surrounding structures were similar to those described in adult patients, and this depends essentially on adult-parasite viability. This localization of the adult worms in pediatric cases was peculiar and distinct from that observed in adult patients, in whom the adult parasites are usually found in extra-nodal lymphatic vessels (Figueredo and Dreyer, 2005).

Most infections are asymptomatic, but the living adult worm progress lymphatic vessel dilation and dysfunction, and the microfilariae also cause swelling, thickening, and discoloration of the skin. Lymphatic dysfunction leads to lymphoedema of the leg, scrotum penis, arm or breast, which can increase in severity as a result of recurrent secondary bacterial infections. However, the presence of living worms in the body is mainly asymptomatic but the death of adult worms leads to glaucomatous inflammation and permanent fibrosis (http://w3.whosea.org/lymphatic/, 17/08/2006). The host reaction to the parasite is considerable and worsen when the worms molt; when the females first

begin to produce microfilariae; and when the worms die and degenerate (http://www.gsbs.utmb.edu/microbook, 06/03/2006) Similarly, immune-mediated pathology in lymphatic filariasis most commonly derives from the lymphatic obstructive consequences of the responses to dead or dying worms in the lymphatic (deVries, 2005), but it is implicated now days that endosymbiont *Wolbachia* of the lymphatic filarial parasite is playing the role in this pathology (Taylor, 2002).

Filarial nematodes harbor intracellular, gram-negative bacteria belonging to the genus *Wolbachia* which are required in the development of filarial nematodes. These bacteria have been observed in various species of filariae, including the main filariasis agents of humans and animals. It has been implicated that *Wolbachia* could play an important role in the pathogenesis of filarial diseases (Bazzocchi, 2001) that characterized by acute and chronic inflammation. The inflammatory responses were thought to be generated by either the parasite, the immune response of the host, or opportunistic infection, but recently, it has been shown that soluble extracts from the human filarial parasite *B. malayi* can induce potent inflammatory responses, including tumor necrosis factor (TNF)-alpha, interleukin (IL)-1beta, and nitric oxide (NO) from macrophages suggesting that extracts of *B. malayi* contain bacterial LPS (Taylor et al., 2000). In contrast to this, they have also observed that extracts from the rodent filaria, *Acanthocheilonema viteae*, which is not infected with the endosymbiotic *Wolbachia* bacteria, failed to induce any inflammatory responses from macrophages, confirming that the source of bacterial LPS in extracts of *B. malayi* is the *Wolbachia* endosymbiont. In addition, proteins of *Wolbachia* have been shown to determine specific IgG responses in animals infected by filariae (Bazzocchi et al., 2001) and some *Wolbachia* molecules (such as LPS) have been shown to stimulate innate-immunity responses, for instance production of cytokines such as IL1, IL6, IL10, TNF-alpha and IFN-gamma by macrophages (Fig. 2). Thus, *Wolbachia* LPS may be one of the major mediators of inflammatory pathogenesis in filarial nematode disease.

It has also been shown that the *Wolbachiae* are associated with severe inflammatory reactions to filarial chemotherapy, being released in to the blood following the death of

the parasite, and in animal models even implicate *Wolbachia* in the onset of lymphodema and blindness suggesting that the endosymbiont bacteria play a major role in the pathogenesis of filarial diseases (Taylor, 2002), although Chirgwin et al. (2003) have shown that *Wolbachiae* may not play a primary role in the formation of lymphatic lesions in mammals chronically infected with filarial parasites. In addition, Keiser et al. (2002) have clearly demonstrated that the endosymbiotic *Wolbachia* play a prominent role in filariasis post treatment reactions, which before have been largely assumed to result from the hosts immune system reaction to the sudden exposure to the large quantities of parasite antigen upon death, implying that additional use of antibiotic may decrease the severity of these reactions.

As indicated in Wikipedia encyclopedia, a large part of the pathogencity of filarial nematodes is due to the host immune response toward their *Wolbachiae* of which the antigens can stimulate host immune responses that may be associated with the development of filarial diseases.

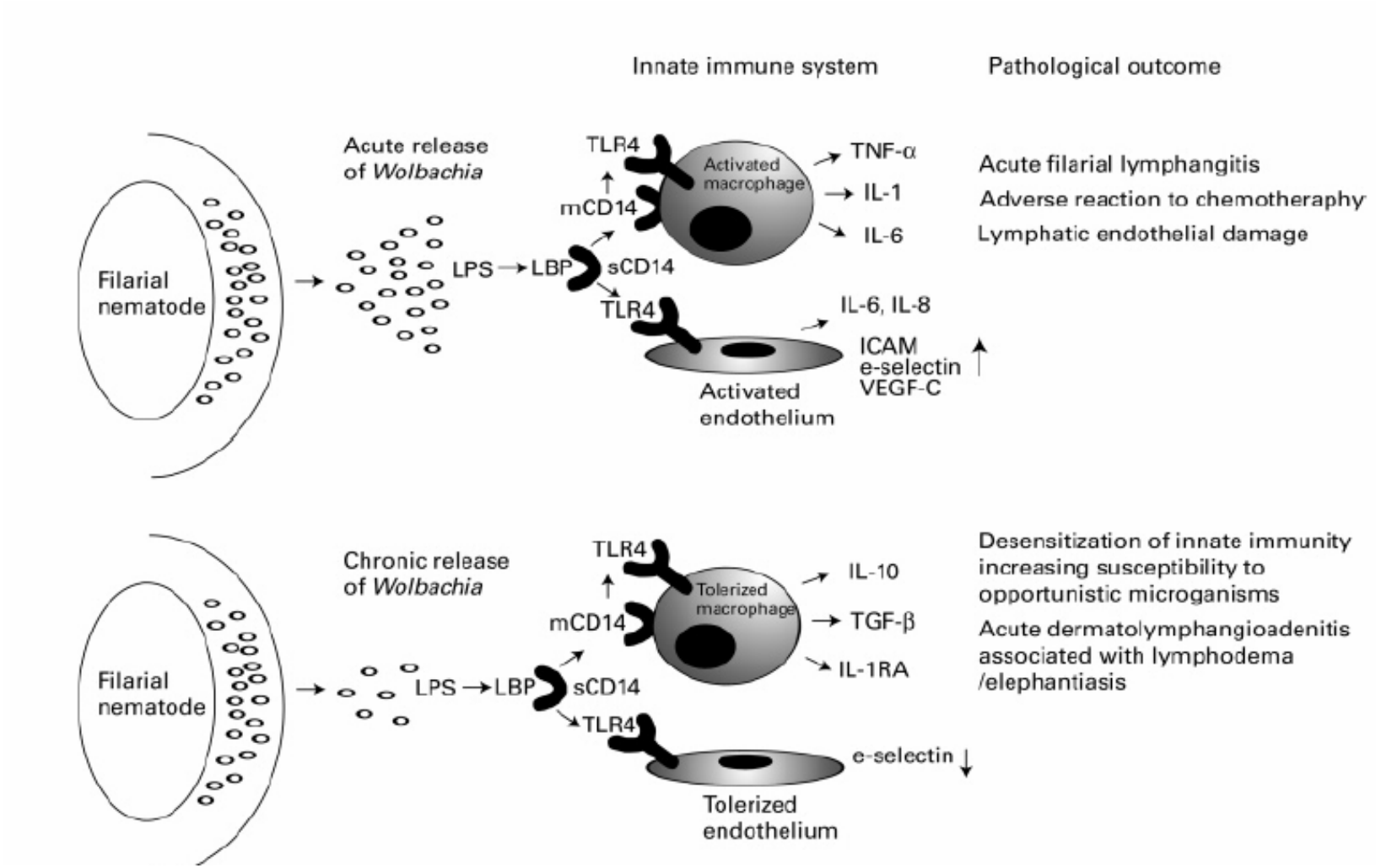

Fig. 2. An overview of the proposed mechanisms, by which *Wolbachia* contributes to the pathogenesis of lymphatic filarial disease

Source: Taylor et al. (2001).

Lymphatic filariasis also causes high social and economic burden to individuals, communities, and the nation (Michael et al., 1996).

1.1.6. Diagnosis of lymphatic filarial infection

A diagnosis of elephantiasis is made based upon a thorough clinical evaluation, a detailed patient history and identification of characteristic symptoms. A variety of tests may be used to determine the underlying cause of lymphatic damage and the subsequent elephantiasis.

The presumptive diagnosis of filariasis must include lymphangitis and lymphodama, but confirmatory diagnosis of bancroftian filariasis relied until recently almost exclusively on the detection and identification of microfilariae in night blood specimens (McMahon et al., 1981 and Nicolas, 1997) Even though it may be very difficult to obtain blood from a patient late at night or during the early morning hours, it is recommended night blood collection as microfilariae concentrated in the peripheral capillaries at these time (Garcia and Brukner, 1997). The reason for this is that, in most geographical areas, *W. bancrofti* microfilariae has a natural periodicity, with highest intensity in the peripheral blood at night and few or none during the day (Simonsen and Meyrowitsch, 1998). The other alternative test is a DEC provocation test, where the suspected patient is given a single oral dose of 50–100 mg of diethylcarbamazine (DEC) is a safe drug, which is normally used for the treatment for lymphatic filariasis, followed by a blood sample 30–45 minutes later: this procedure can "flush out" microfilaria into the peripheral blood during day time and has a sensitivity that is almost comparable to that of night blood surveys (Sasa et al., 1963 and Manson-Bahr et al., 1972). Tests based on detection of specific *W. bancrofti* circulating filarial antigen (CFA) have been highly successful and a number of kits have recently become commercially available. The high specificity and sensitivity of this test has been confirmed in a number of studies (More and Copeman et al., 1990; Chanteau et al., 1995; and Weil et al. 1997). More recently, ICT diagnostics produced a simple immunochromatographic card test (AMRAD ICT) and (NOW ICT) for the detection of circulating filarial antigen in serum and whole blood specimens (Weil et al., 1997; Phantana et al., 1999; (http://www.binax.com, 16/04/20008). In this test, antigen capture

is accomplished by a monoclonal antibody with high sensitivity and specificity to adult *W. bancrofti* antigen (Weil et al., 1997). The NOW ICT filariasis is an in vitro immunodiagnostic test for the detection of *W. bancrofti* antigen in the whole blood, serum or plasma using a polyclonal antibody and a monoclonal antibody specific for *W. bancrofti* (http://www.binax.com, 16/04/20008). The test is rapid and requires no equipment, which makes it suitable for use in many endemic areas in the developing world. It is known that filarial elephantiasis can result if the infection is left untreated. Limited treatment modalities exist and no vaccines have been developed.

The differential diagnosis between filarial elephantiasis and non-filarial elephantiasis can be made clinically, considering the swelling of the toes and itching at the base of the toe clefts as the first symptoms of the non-filarial type, together with burning of the lower legs in the bed at night. More over, in non-filarial elephantiasis only the lower limbs, are involved (de Lalla et al., 1988). Therefore, in non-filarial elephantiasis endemic areas, diagnosis of the condition can often take place in the community setting, where laboratory facilities are not available; hence, clinical examination is a valid means of diagnosing the case (Desta et al., 2007).

1.2. Non-filarial elephantiasis (Podoconiosis)

Non-filarial elephantiasis (non-filarial elephantiasis) clinically defined as a chronic & asymmetrical obstructive lymphopathy of the lower legs, occurring in barefooted peoples living on fertile clay soil (Price, 1974b).

The history of non-filarial elephantiasis is long, and can be traced through medical and non-medical writings. The condition is referred to by writers in Persia, Tibet, South America and India as well as Ethiopia. The history may be”Beginning from the time of the Roman Empire, travelers recorded anecdotes about people with progressive swelling of the feet. A more detailed reference to ‘swollen legs’ appears in the Tibetan translations of a fourth century revelation originally recorded in Sanskrit as the second book of Gynd-bzhi. However, it was not until about AD 905 that the Persian physician Rhazes first distinguished elephantiasis ‘of the Greeks’ (lepromatous leprosy) from that ‘of the Arabs’

(da-al-fil or ‘elephant sickness’, most probably non-filarial elephantiasis). The chief site of this disease is from the bending of the knee downwards to the ankle; the leg is swelled to a great degree, becoming one size from bottom to top, and gathered into circular wrinkles. It should seem that the black color of the skin, the thickness of the leg, its shapeless form and the rough tubercles or excrescences, very like those seen upon the elephant, gave the name to this disease
(http://www.ethpress.gov.et/Herald/articleDetail.asp?articleid=2717, 23/04/2007)

The noticeable observation of non-filarial elephantiasis is dated back to Augustine’s report in 1957 that stated the assumed relationship between filariasis and elephantiasis lacks scientific foundation commenting that information is truly needed on the extent and severity of elephantiasis in tropical areas including Ethiopia where no form of the filarial infection is known to exist while he had reported on filariasis to an expert committee of WHO as cited by Tekola et al. (2006).

Non-filarial elephantiasis is found in highland areas of tropical Africa, Central America and north-west India. Areas of high prevalence are documented in Uganda, Tanzania, Kenya, Rwanda, Burundi, Sudan and Ethiopia, to the east side of Africa (Price, 1976b), and in Equatorial Guinea, Cameroon, the islands of Bioko, Sao Tome & Principe (Ruiz et al., 1994) and the Cape Verde islands to the west. The condition has been reported in the Central American highlands from Mexico south to Ecuador. In the past, non-filarial elephantiasis was common in North Africa (Algeria, Tunisia, Morocco and the Canary Islands) and Europe (France, Ireland and Scotland), but is no longer found in these areas after shoes wearing become routine (Davey et al., 2007). Within Ethiopia, non-filarial elephantiasis is present over approximately one fifth of the land surface, excluding the Danakil depression, the Blue Nile Basin, the Rift Valley, the Awash valley, and the south-east lowlands”
(http://www.ethpress.gov.et/Herald/articleDetail.asp?articleid=2717, 23/04/2007

Soil borne minerals (rather than parasites, bacteria or viruses) are the primary cause of non-filarial elephantiasis which is a disorder caused by the absorption of minute mineral

particles from the soil through the feet of barefoot individuals. It is believed that the mineral particles cause an immune system response eventually resulting in the formation of inflammatory masses of nodules (granulomas) in the lymph vessels of the feet and legs (http://www.cigna.com/healthinfo/nord689.html, 24/04/2007). Specifically, non-filarial elephantiasis is caused mostly by silica and alumino-silicate (Price and Henderson 1974), and is characterized by bilateral asymmetrical swelling of the feet and lower legs, which is mostly seen in peoples of bare footed especially farmers working in well defined fertile volcanic soils of high land zones of Africa, Central and South America, and Indonesia, and also in the lowlands irrigated by rivers from these highlands (http://www.meb.uni-bonn.de/dtc/primsurg/docbook/html/x11094.html,7/3/2006). Non-filarial elephantiasis (non-filarial elephantiasis) is also occurring in individuals exposed to red clay soil derived from alkalic volcanic rock (Price 1976a).

Although rarely lethal, non-filarial elephantiasis is a chronic and debilitating disorder, which is a considerable public health problem in at least ten countries in Tropical Africa, Central America and North India (Price, 1990). How ever, Ethiopia does present the largest concentrations of non- filarial elephantiasis of the legs reported in Africa. It was calculated by Oomen (1969), who described the exclusion of a filarial etiology, that there were more than 100,000 cases in Ethiopia at the time. Similarly, it has been investigated by Price (1974b) that the prevalence of endemic elephantiasis of the lower legs in relation to the red soil on which the barefooted population lives become higher than the other types of soil showing prevalence that falls from a maximum of 6.92% on the red soil area, to 2.96% at the limit and to 0.98% in two directions within 25 km of the edge of the red soils.

Price (1974) also suggested that a chemical irritant absorbed in colloid form through the bare feet is an etiological factor in the disease. It is a geochemical disease induced by the absorption of ultra fine silica particles from the volcanic clay soil through the skin of the feet in susceptible individuals leading to a progressive obliterative endolymphangitis (Price, 1985; Claire, 2005). On the other hands, it is suggested that the high proportion of iron and other transitional metals from soils of volcanic rocks may also be important as

irritant or toxic to the lymphatic vessels of the legs after absorption (Price, 1976a). Similarly, Claire, (2005) has noticed that the disease is induced by the absorption of ultrafine silica particles from the soil (Konia) through the skin of feet (podos) in susceptible individuals, leading to a progressive obliterate endolymphangitis. The presence of microparticles of clay was also observed by Blundell *et al.* (1989) in the dermis of the foot of the patients, especially in the phagosomes of macrophages or in the cytoplasm of other cells. Consequently lymphatic vessels fail to conduct lymph to the nodes producing permanent deposits of silica in the dermal tissues. Price (1977) has also indicated that the lesion responsible for the irreversible elephantiasis of the lower legs is due to the blockage of the lymphatic distally.

Intralymphtic silica provoked an immediate and intense macrophage reaction with later fibrosis both within lymph vessels and to a lesser extent within lymph modes in experimental animals (Fyfe and Price, 1985). Similarly, Spooner and Davies (1986) have investigated the physical composition of soils from both endemic and non-endemic areas of Ethiopia and their effect on the viability of macrophages using in vitro systems. Soils (contents of silicon and aluminium) of the two areas were avidly phagocytosed by the macrophages as a result the cell shape was changed from approximately circular to spindle shape implying that soils were cytotoxic towards the macrophages killing large amount of cell within specified time. This supports the epidemiological studies implicating soils as the cause of non-filarial elephantiasis.

Non-filarial elephantiasis is a non-communicable disease producing lymphodema of the lower limbs; it affects predominantly barefoot agricultural workers in tropics (Claire, 2005). It is also pointed out, for many years this disease has not been widely recognized as distinct from lymphatic filariasis and yet it may affect 10% of populations in volcanic tropical highlands. It produces considerable morbidity associated with lymphodema, impacting on economically productive age groups. It has also been commented by Davey and Newport (2007) that non-filarial elephantiasis is a common, but barely recognized, non-communicable tropical disease, and the communities in which this disease occurs are among the poorest in the world and the disorder intensifies the existing economic and

social burden. It is also implied that non-filarial elephantiasis is more common than HIV infection in areas where this disease is best described in Ethiopia (Desta et al., 2003).

Not all individuals exposed to red clay soil (irritant soil) develop non-filarial elephantiasis, and family clustering has long been observed. Genetics studies show high heritability of the trait, and segregation analysis suggests the presence of an autosomal codominant major gene conferring susceptibility to the non-filarial elephantiasis (Davey et al., 2007). This implies that the host genetic factors are important determinant of susceptibility to non-filarial elephantiasis and supports the hypothesis that stated as “the disease occurs in genetically susceptible individuals on exposure to an environmental element in the soil”.

At a varying interval after the initial stage, the swelling progresses up the foot and lower leg, either insidiously or as a result of a series of acute episodes which is a features of progressive phase of the disease, and the two legs are affected independently and asymmetrically so one may progress while the other remains quiescent (Price, 1974a). He had also indicated that the asymmetry of the leg swelling in a healthy person is a diagnostic feature of this disease.

Although there are no general symptoms observed among the patients of this disease, Price (1974a) has out lined three main common symptoms that may indicate the beginning of the disease. These are plantar oedema, splaying of the forefoot, and prominence of skin wrinkles. All patients had been bare footed until the onset of disease. This disease is entirely preventable as it has been eradicated from Scotland, France, and the Canary Islands after footwear become routine Price (1990).

1.3. Elephantiasis in Ethiopia

“In Ethiopia, the adventurer James Bruce used his amateur medical skills to good effect when traveling in the 1770s. He gave a graphic description of the elephantiasis he saw in Gondar, which is quoted in Richard Pankhurst’s Introduction to the Medical History of Ethiopia:

Records describing the condition next arise from two missions in Ethiopia in the 19th century. The first was the British diplomatic mission of 1841-42, when 717 patients with various conditions (predominantly syphilis) were treated, of which six had a diagnosis of elephantiasis. The second mission was that of the Russian Red Cross in 1896. Twenty-five cases of elephantiasis were treated in Harar, and twenty-three in Addis Ababa, of a total of 7819 and 5237 in each city, respectively. There is no way of knowing whether the cases treated by either mission represented filarial or non-filarial cases.

Through the eighteenth and nineteenth centuries, the disease process of elephantiasis was gradually elucidated through study of the lymphatic system in affected people. A Dr Hendry, practicing in Barbados, considered the problem of elephantiasis to be "seated in the lymphatic system". Investigators in Brazil and India demonstrated the role of filarial parasites in elephantiasis, and for a time it was concluded that all elephantiasis was filarial. Towards the end of the nineteenth century, the discrepancy between distribution of elephantiasis and distribution of filaria in North Africa, Central America and Europe prompted revision of this theory. Detailed description of patients living in areas of Guatemala too high for filaria to be transmitted suggested that their elephantiasis was an endemic condition closely associated with walking barefoot. A physician named Robles performed many tests for filarial infection, and all were negative. He considered that there was "An immense difference between those who wear shoes and those who do not". Progress in recognizing the international distribution of non-filarial elephantiasis came as the term 'idiopathic lymphoedema' was suggested in place of the local terms 'verrucosis lymphatica' in Kenya and 'mossy foot' in Ethiopia. The British physician Ernest Price, who practiced at the Zenebework Memorial Hospital and the Department of Medicine at Addis Ababa University did extensive work into the causes of the condition which led him to propose the term 'non-filarial elephantiasis' (from the Greek for foot: podos, and dust: konos), which has gained widespread acceptance" (http://www.ethpress.gov.et/Herald/articleDetail.asp?articleid=2717, 23/04/2007), hence the present name of this disease.

An association between non-filarial elephantiasis and walking barefoot was suspected by Robles in Guatemala City at the end of the nineteenth century. However, it was not until

Ernest Price superimposed maps of disease occurrence in Ethiopia onto geological surveys that persuasive evidence of a link with red clays associated with alkalic volcanism was provided. Significant differences in the prevalence of non-filarial elephantiasis were measured either side of the natural limits of red soil in the country. Further geological study suggested that the climatic factors necessary for producing these irritant reddish clays include high altitude (over 1,000m above sea level), and seasonal rainfall (over 1,000mm annually) (Price, 1976a). These conditions contribute to the steady disintegration of lava and the reconstitution of the mineral components into silicate clays. Particles of the elements common in these clays (aluminium, silicon, magnesium and iron) penetrate through the skin, particularly through injuries of the feet and have been demonstrated inside lymph node cells in the legs of people living barefoot on the clays (Price and Bailey, 1984). Experiments have also shown that these particles are very irritant to lymph vessels in animals, and can completely block them (Fyfe and Price, 1985). If lymph vessels become blocked, the lymph fluid that keeps the body cells moist and active no longer circulates, and pools in the legs and feet, first causing mild swelling, and eventually the full elephantiasis.

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In one form or another elephantiasis is seen throughout the tropical world, but the etiology varies. Even though there was no firm evidence for the presence of *W. bancrofti* in Ethiopia, many textbooks placed the country in the sub-Sahara endemic zone for quite some time. However, the microfilariae of *W. bancrofti* had been identified and ascertained in 1971 by McConnell and Schmidt (1973) and McConnell et al., (1976) only in southwestern low lands of Ethiopia, and also clinico-epidemiologically studied by Jemaneh and Kebede (1995) in this area though they explained that the parasite has not caused the elephantiasis in the microfilariamic persons.. Similarly, it has been shown that the symptoms of the disease are not much manifested in the area where the parasite is prevalent although hydrocele and/or scrotal elephantiasis have been reported (McConnell and Schmidt, 1973 and Jemaneh and Kebede, 1995) suggesting that the lymphatic filariasis may be relatively new to the area. They have also indicated that lymphatic filariasis is a public health problem in the surveyed area. In contrast, most of the studies underwent in Ethiopia on the elephantiasis have concluded that the content of red clay

soil of volcanic origin soils are the main etiologic agents of the disease (Price, 1974b; Price and Henderson, 1978 and 1979; Birrrie et al., 1997; and Desta et al., 2003). Endemic non-filarial elephantiasis of the lower legs in Ethiopia is related to the distribution of red clay soil derived from volcanic rocks, particularly basalt (Price, 1976b).

Elephantiasis in Ethiopia is of complex etiology. In many instances it almost certainly is not of filarial origin (Price, 1973), and in other it may be associated with presence of onchocerciasis (Oomen, 1969). Nevertheless, Price (1974b) has critically examined elephantiasis in persons from high land Ethiopia, and he too concluded that filariasis is not a cause of the infection. Price and Henderson (1979) have shown the numerous particles of colloid-size and amorphous elements predominantly of Si, Al and Fe in the lysosomes of the macrophages in the gland of femoral lymph nodes of barefooted Ethiopians using electron microscopy, electron-density, diffraction analysis, and elemental microanalysis of the X-ray spectrum. They also observed that in elephantiasis case some particles contain silicon alone, presumably silica supporting the hypothesis that the disease is silicosis of the peripheral lymphatics of the lower limbs (Ziegler, 1993). In contrast, Formel et al. (1993) have identified Zr and Be elements that found in clay rich in colloidal silica particles (at Ochollo area, in Rift valley), are highly abrasive to skin, and they doubtlessly explained that these elements are a factor involved in the development of lymph node sclerosis leading to elephantiasis. These elements are known for their ability to induce granuloma formation in the lymphoid tissues of the affected person upon long period of time exposure. Furthermore, Price et al. (1981) have revealed that the residual silica is recognized as an agent toxic to macrophage; the fibrosis of the afferent lymphatic of the feet is a possible result of the passage of damaged macrophages containing alumino-silicate particles absorbed through the feet. Although they have observed that there is no elemental difference between the nodes of elephantiasis and of non-elephantiasis, but clinically greatly enlarged nodes in diseased persons indicated a heavier total mineral load in elephantiasis cases. They also interpreted the difference in fibrotic response depends on tissue variability, as in the lung disease of miners.

In a population-based study of non-filarial elephantiasis in two resettlement schemes in western Ethiopia, Kloos et al. (1992) have shown that the prevalence rates are generally higher in males than females and increased with age, indicating sex differences in occupationally linked trauma to the feet and the cumulative effect of long-term exposure to volcanic soils.

Most people are affected by non-filarial elephantiasis (non-filarial elephantiasis), the non-communicable disease that causes elephantiasis of the lower limbs in bare footed people in tropical high lands including Ethiopia. Like lymphatic filariasis, it has immeasurable emotional and economic effects on the patients in the community though the specified prevalence of the disease is not known in Ethiopia. However, Claire (2005) have estimated that non-filarial elephantiasis may affect about 10% of populations in volcanic tropical high lands indicating that the disease has not been widely recognized as distinct from lymphatic filariasis for many years. A comprehensive study undertaken in Wolaitta zone, SNNP region of Ethiopia by Desta et al. (2003) identified a mean zonal prevalence of 5.46% of the population with 64% being in the economically productive age group as the lower legs are progressively involved during the course of several months to years, but the thigh is rarely affected. As reviewed by Davey and Newport (2007) above 11 million people (18% of the national population) live in endemic areas in Ethiopia, and between 500,000 and one million people are affected nationwide.Economically, Tekola et al. (2006) have estimated that total direct costs of non-filarial elephantiasis to be equivalent of US $ 143 per patient per year and the total productivity loss for a patient to amount to 45% of the total working days per year, causing a monetary loss equivalent to US $ 63 per year. This shows the enormous economic impact of non-filarial elephantiasis in affected area.

Elephantiasis is a significant public health problem throughout tropical developing countries. Although it is not a major cause of mortality, elephantiasis causes morbidity that has devastating social and economic significance for both individuals and the community.

Likewise, in Midakegn (the newly established) district of West Shoa zone, which is located in northwestern part of the zone, elephantiasis the causes of which are not known was observed. Although this disease is common in the middle and low ecological zone of this district, no study has been documented so far probably due to lack of accessibility and other infrastructure. In addition, most of the cases of elephantiasis were non-specifically reported but together with other acute feverile episodes. As a result, the physical, socioeconomic, and psychological impact on the infected persons and the etiology of this disease in the district was not known. The present study is intended to determine the etiology of elephantiasis in Midakegn district so as to enable institution of appropriate control measure

2. Objectives

2.1. General objective

- To investigate the prevalence and determine the etiology of elephantiasis in the Midakegn district, western Shoa zone, Oromia region.

2.2. Specific objective

- To identify the etiology of the elephantiasis in the Ganbela and Tullu Eteya kebeles, Midakegn district
- To assess disease prevalence in relation to sex and age.
- To assess the possible familial predisposition to the disease
- To assess the effect of shoe wearing on elephantiod swelling, the legs

3. The study population and methods

3.1. The study area

The study was carried out in two kebeles (Tulu Eteya and Ganbela) of the Midakegn district, which is located in North West of West Shoa Zone, Oromia region, and is located approximately 230 Kms away from Addis Ababa in the central part of the Ethiopia and 98 Kms from Ambo town. The district was established in 2002, formerly found as one part of Cheliya district. The administrative town of the district is Ballami, which is formerly known as Hamus gebeya (or Gaba kamisa in Afan Oromo).

The topographical features of the district are full of hills, gorges, and different ups and down observed in the country in general. Because of this features the district is exposed to erosion in summer season losing much but unknown amount of the fertile soil esp. from the highland areas of the district this eroded soil get entry to Blue Nile through the tributary river known as Hanonu passing via the Gnemer forest where different wild lives like lion, deer, tiger, and warthog are living and that found in between Gendeberet and Midakegn district.

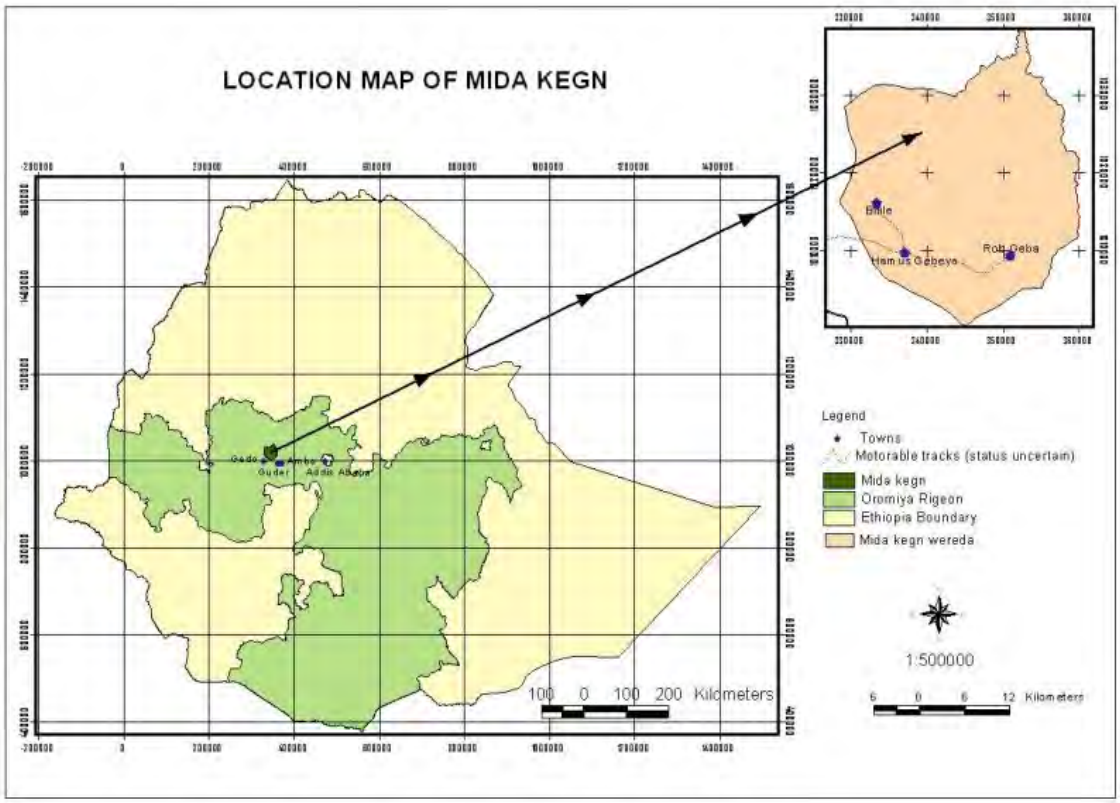

Fig. 3. The location map of Midakegn district with in Oromia region of Ethiopia

The road to Midakegn district is junction to the right hand side from the road to Fincha sugar factory at the place known as Tullu Lencha. The road that leads to Fincha is in turn junction from the main road to Welega at Gedo town, the administrative office of Cheliya district.

According to the office of agriculture and development office of the district, the district comprises the low land (Kola), middle ecological zone (Weinadega), and high land (Dega) ecological zones approximately ranging from 1320m to 3500m a s l. The district is demarked by East Welega zone in Western and Northwest; Gindeberet district in Northern; Ambo district in Eastern; and Cheliya district in Southern. The district has different streams (rivers) that drain to Blue Nile River. These streams erode the fertile soils of main farmlands on which different cereal crops and oil seeds are yielding. Two of these streams pass through the present study area.

The study was undergone, following the Wangalle stream, which demarked East Welega zone and the Midakegn district in particular and the west Shoa zone in general, and Walda stream, which originates from the highland of the district and passes in between the two kebeles (Ganbela and Tullu Eteya) in which this study was focused on as the people were highly affected by elephantiasis relative to the other kebeles of the same ecological zone of the district. These two kebeles are located in northwest of the district having an elevation range 1320m to 1640m a s l; the inhabitants are Afan Oromo-speaking group, and live in rural agricultural areas.

3.2. The study Population

The study populations of the area are the residents of the two kebeles (Ganbella and Tullu Eteya); none of them are from the other kebeles although the disease is also observed in higher altitudes and within equivalent altitudes.

The two kebeles are found side by side in northwest of the district approximately 12 Kms away from the district's office The populations in both kebeles are farming on fertile red, brown, sandy, and clay soil lands yielding mainly maize, sorghum, and teff. The soil in the area is reddish-brown clay like, which, when wet is strongly adherent to the skin during rainy season and such type of soil is primarily located in the mid-latitudes (Price, 1976b). Although they are engaged in crop-livestock mixed agriculture, they are not food self-sufficient and some time they are dependent on donation from government. Because of this some of the residents are also involved in another additional works such as weavers, pottery and smith to overcome the food problem. This income again is not enough to buy footware even the ordinary plastic shoes or sandals. Further more, the residents of this area had limitation with raising livestock due to the presence of animal trypanosomiasis until recently.

The suspicion that lymphatic filariasis could be the case in the specified study area of Midakegn district, west Shoa zone, where high effect of elephantiasis had been observed led us to the present study. However, the district's health authorities had not considered elephantiasis as a serious public health problem. Consequently, the appropriate report or

data of this disease was not made in the district although it is ranked as the third level, together with the other acute feverile, next to diarrhea and malaria among the top ten diseases of the district.

3.3. Methods

The study was a cross sectional survey for the elephantiasis. However, Ganbela and Tullu Eteya kebeles of the district were selected on the basis of the number (estimated) of infected individuals coming to the nearby markets and other social services, and information given from the health professional assistants of the district. Based on this information, house-to-house survey was made (Birrie et al., 1997 and de Lalla et al., 1988) on the whole population of age >14 to attain individuals with elephantiasis within the two selected kebeles by the researcher and health assistants.

After explaining the purposes of the study using Afan Oromo (the local language) to the kebeles` leaders, later to the residents on meetings and other social services, and obtaining their permission, the house-to-house surveying and recording individuals >14 years was done in the two kebeles (Birrie et al., 1997 and Onapa et al., 2001).

After some periods of time, the populations of the two kebeles were examined both physically and clinically during the house-to-house survey and at the health centre of the district, respectively. After oral consent was obtained from the participants, the individuals with overt elephantiasis case were assigned for parasitological and immunological tests and also for interview.

3.3.1. Blood specimen collection for parasitological diagnosis

The finger prick blood thick smear is by far the easiest technique available for detecting Mf although the technique has less accuracy when the Mf density is low (Partono et al., 1973). From each participant, the finger-prick blood was collected at night, between 10 p.m. and 2 a.m. (Garcia and Brukner, 1997) at Midakegn district health centre. The participant’s left hand index finger was cleaned with denatured alcohol and then punctured using a sterile lancet and 20µl fresh blood sample was collected in a calibrated

capillary tube and then the blood placed onto cleaned microscopic slide to form thick blood smear (Partono et al., 1973 and WHO, 1987). After 15 minutes, the prepared blood film was laked in saline water (0.85%) to dehaemoglobinize; then the slides were fixed with pure methanol; and were stained in Giemsa stain solution (dilution 1 in 20) for 15–20 minutes (Denham et al., 1971 and WHO, 1987). Finally, these slides were examined microscopically at biomedical science laboratory, Addis Ababa University. Test results were recorded coinciding with the registration number given during house-to-house survey in the data sheet.

3.3.2. Blood specimen collection for serological diagnosis

Following the parasitological examination, after some periods, blood specimen were collected from randomly selected 48.78% of the parasitologically examined participants and examined for circulating filarial antigens with the NOW ICT card test for whole blood specimen following the instructions given by the manufacturer (http://www.binax.com 16/04/20008) as confirmatory test for the etiology of elephantiasis. Because the ICT is very sensitive and specific to Lymphatic Filariasis, detecting the infection within minutes, at any time of day, without the need for laboratory facilities. It is manufactured by Binax Inc., a global diagnostic tool company, which is providing test kits at cost to the Global Programme to Eliminate Lymphatic Filariasis.

3.3.3. Assessment of knowledge, attitude, and practice about elephantiasis

In the next day morning, all individuals, who were participated in parasitological examination, were taken to a screened examination room individually and were interviewed using an open ended questions for their life style; bed net usage in the household; time of disease on set; soil types on which they work; shoes usage; the size change of the swelling after wearing shoes; the presence of this disease within their family; and their view on the elephantiasis; and the participants were also interviewed for the presence of scrotal swelling or hydrocele by the local health professionals to elucidate the etiology of the disease.

3.4. Data analysis

Data were entered into a computer and statistical analysis was carried using SPSS software packages (version 13.00) for entire study (http://www.spss.com/, 12/09/2007). The data were analyzed using Pearson Chi-square tests (χ^2). Values were considered to be statistically significant when the p-valve obtained was less than 0.05.

4. Results

Primarily physical observation was made on 1656 people, of which 916 are living in Ganbella and 742 in Tullu Eteya kebele (local census) and of this population 754(45.5%) are females, during house-to-house survey for the case in the two kebeles of the district. Of the total population 123 (7.42%) individuals with overt chronic elephantiasis of lower legs were identified (excluding individuals those who were not found at their home during the survey) for further study (for parasitological and immunological tests and for interview). This showed that the prevalence of the case seems to be 7.42% in the area. Of the 123 identified participants 56 (45.5%) were females. The mean age is 43.4 years (range 14–70 years), and nearly both males and females were equally affected.

4.1. Clinical observation

The distribution of elephantiasis within the two kebeles with relation to sex of the participants is presented in table 1. One hundred sixteen (94.30%) of the participants, 62 (50.4%) males, 38 (30.90%) from Ganbella and 24(19.50%) from Tullu Eteya, and 54 (43.90%) females, 28(22.76%) from Ganbella and 26(21.13%) from Tullu Eteya, were clinically observed with both leg (bilateral asymmetric) elephantiasis; the rest were either of left or right leg only (Table 1). However, no individual were observed with thigh elephantiasis, scrotal elephantiasis, and hydrocele which are the characteristics indicators of lymphatic filarial elephantiasis.

Peoples that are affected their either left or right leg are seems of short period of time of infection. But one old participant remains with single leg swelling without any practice against the disease.

Table 1. The distribution of elephantiasis Ganbella and Tullu Eteya, Midakegn district, western Shoa zone, 2006 – 2008

		Elephantiasis			
Kebele	Sex	Both legs	One leg	Total	p-value
Ganbela	M	38 (30.90)	3 (2.44)	41 (33.34)	0.916
	F	28 (22.76)	2 (1.63)	30 (24.39)	
Tullu Eteya	M	24 (19.50)	2 (1.63)	26 (21.13)	0.149
	F	26 (21.13)	–	26 (21.13)	
Total		116 (94.29)	7 (5.70)	123 (100.0)	

Numbers in parenthesis is percentage (%)

p-value comparing prevalence between male and female in the two kebeles Ganbella and Tullu Eteya

The majority of the participants affected by elephantiasis were of the productive age groups that are found within 21 – 60 years age group (Table 2; Fig. 3).

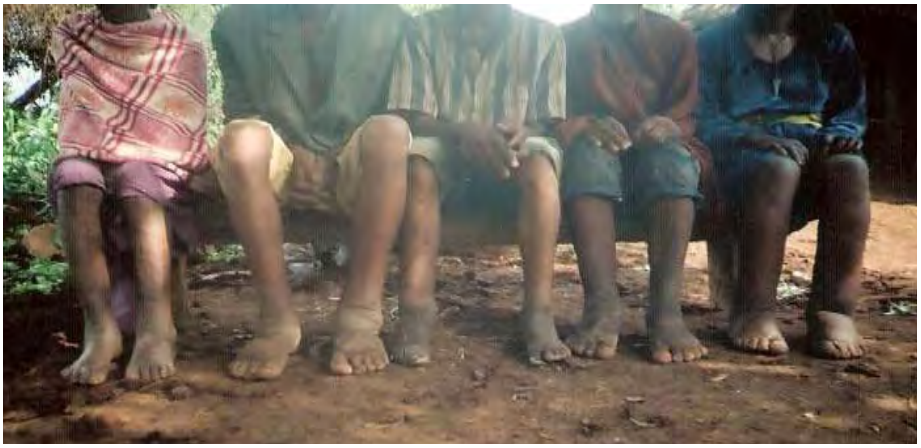

Fig. 4. The productive age groups (21 – 40) from the same village affected their both legs, in Ganbela kebele, Midakegn district, west Shoa zone, 2007

Table 2. The distribution of elephantiasis within age groups and sex in Ganbela and Tullu Eteya kebeles, Midakegn district, West Shoa zone, 2006 – 2008

Sex	Age Range (group)				Total	p-value
	<= 20	21 - 40	41 - 60	> 60		
M	4 (3.22)	25 (20.16)	24 (19.35)	14(11.29)	67 (54.50)	0.0075
F	8 (6.45)	23 (18.55)	24 (19.35)	1 (0.80)	56(45.50)	0.0055
Total	12(9.67)	48 (38.71)	48(38.71)	15(12.09)	123(100)	

Numbers in parenthesis is percentage (%)

p-value comparing among the age group with relation to sex

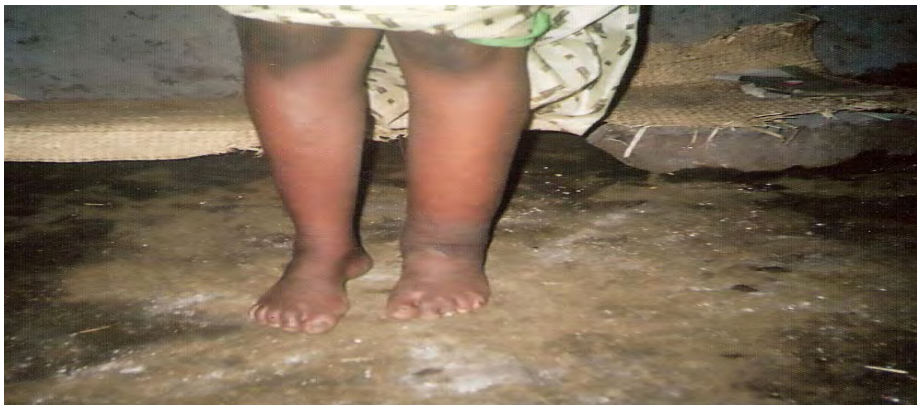

Fig 5. The beginning of elephantiasis of Left leg affected girl of 20 years old living in Ganbela kebele, Midakegn district, 2007

Fig.6. A woman of 28 years old chronically affected and disabled, living in Ganbela kebele, Midakegn district, west Shoa zone, 2007.

4.2. Parasitological diagnosis

The parasitological diagnosis of the night collected blood specimen from the entire identified participant were turned to be negative for microfilariae of the *W. bancrofti*. In addition, our clinical examination indicated all of the identified participants had elephantiasis of the lower leg(s).

4.3. Serological diagnosis

Sixty (48.78%) individuals of those clinically and parasitologically examined were also diagnosized serologically for circulating filarial antigen of *W. bancrofti* using immunochromatographic test (ICT) kit from the finger brick blood specimen collected in day time. All of the tests for filarial antigen also turned to be negative like that of parasitological examination. The control lines developed as required indicate that the cards were valid.

4.4. Knowledge, attitude, and practice about elephantiasis

About 57.7% of the participants were from Ganbella kebele and the rest are from the Tullu Eteya (Table 1). All the participants are the residents of the area; the duration of onset of illness (swelling of feet or leg) mostly ranged from 5 – 49 years although some of them do not know the real time of onset of the disease; however, most participant 43(35.0%) of the elephantiasis fall within the range of 10 – 19 years of onset (Table 3).

Although lymphodema (swelling of lymph node) is common when the illness randomly onset, permanent lymphodema was not observed among the individuals responded. Probably, the random swelling of lymph node may be related to secondary infection or supper infections.

Table 3. Elephantiasis in relation to duration of onset of the disease in study participants of Ganbella and Tullu Eteya kebeles, Midakegn district, West Shoa zone, 2006 – 2008

	Duration of elephantiasis onset (year)						
Elephantiasis of	< 5	5 -9	10 - 19	20 - 29	30 - 39	40 -49	p-value
Both legs n =116 (94.30)	7 (5.7)	16 (13.0)	40 (32.5)	35 (28.5)	15 (12.2)	3 (2.4)	0.18
Single leg only n =7 (5.70)	1 (0.8)	2 (1.6)	3 (2.4)	—	—	1 (0.8)	
Total	8 (6.5)	18 (14.6)	43 (35.0)	35 (28.5)	15 (12.2)	4 (3.3)	

Numbers in parenthesis is percentage (%)

p-value comparing elephantiasis case among the range of duration of onset of elephantiasis

Some families 37(30.08%) of the total participants replied that one or more of their sons, daughters, or their relatives have been affected by the disease. The affliction was mostly observed in sons and daughters of the affected mothers and/or fathers.

Majority of participants 74 (60.16%) responded that they had never worn shoes at any time in their life and that the first sign of the illness is an itchy swelling of the toes and sole of foot. However, among those who wore shoes after the disease set on, 38 (30.89%) individuals got relief and/or got reduction in the size of swelling of their lower legs (Table 4). Interviewed about their knowledge of the cause of elephantiasis. The majority of the respondents did not know about it, while some said that elephantiasis was caused by some kind of a worm in the stream they use, and few blamed poor living condition and evil spirits.

Table 4. Reduction of elephantiod swelling in shoe-wearing individuals of Ganbela and Tullu Eteya kebeles in Midakegn district, Western Shoa zone, 2006 – 2008

	Shoe- wearing habit				
Respondents with Elephantiasis of	Never wore shoes	Wore shoes after the disease onset		Total	p-value
		Swelling not reduced in size	Swelling reduced in size		
Both legs	73 (59.3)	11 (8.9)	32 (26.0)	116 (94.3)	0.005
One leg	1 (0.8)	___	6 (4.9)	7 (5.7)	
Total	74 (60.2)	11 (8.9)	38 (30.9)	123 (100.0)	

Numbers in parenthesis is percentage (%)

p-value comparing prevalence in reduced and not reduced swelling of elephantiasis after shoes wore by the patients

5. Discussion

By using parasitological, serological, clinical examinations, and interviews, this study has shown the etiology of elephantiasis prevalent in the two kebeles to be non-filarial.

Prevalence of elephantiasis of the present study area (7.42%) was found to be as high as, or even higher than those described in other endemic areas for both lymphatic filarial and non-filarial elephantiasis of Ethiopia (Desta et al., 2003; Birrie et al., 1997; Jemaneh and Kebede, 1995; Mengistu et al., 1987; and Price, 1974a), but less than that reported by Kloos et al. (1992). The higher rate may be due to the common practice of walking distant and farming bare foot on soil that may have high levels of etiologic agents as the main source of income of the area is agricultural activities and due to the fact that frequent changes in the weather condition results in the clay soil formation from the volcanic rocks (Price et al., 1981).

Unexpectedly, however, no hydrocele and scrotal elephantiasis were seen. Furthermore, there was no indication of filarial infection in the thick blood smear such as microfilariae detection in the participant's blood sample. The absence of microfilariae of *W. bancrofti* in the blood specimen, the limitation of swelling to the lower legs and feet though the lower leg elephantiasis of filarial cases are present elsewhere (http://www.gsbs.utmb.edu/microbook, 06/03/2006), and the absence of filarial antigen detection in the ICT test suggest that elephantiasis in the district is not of filarial origin, but rather could be a manifestation of endemic non-filarial elephantiasis. This agrees with most studies done in East Africa (Price, 1974a and Formmel et al., 1993)

In the present study, the study participant has no any symptom of hydrocele, which is the most common clinical manifestation of chronic *W. bancrofti* infection in males of endemic areas of the world including Ethiopia (Jemaneh and Kebede, 1995 and Michael et al., 1996).The main clinical manifestation of lymphatic filariasis such as hydrocele and swelling of local glands observed in southwestern Ethiopia, Gambella region by Jemaneh and Kebede (1995) were completely not observed clinically among the present study participants of both kebeles of Midakegn district.

Other epidemiological observation supporting the non-filarial etiology of elephantiasis in the study area is the red tropical clay soil of the area, very rich in silica, with a probable volcanic origin (Price, 1974b) and the fact that the participants had never worn shoes since childhood in their life. Evidence for an association of endemic non-filarial elephantiasis of the lower legs with areas of red clay soils around volcanoes has been noted in several studies of different countries in the world, especially in tropical Africa. It is known that the altitude governs rainfall and temperature and thus governs the type of soil formed. The soil produced from these rocks is rich in colloidal iron oxide, alumina and silica, to which a number of metallic ions are adsorbed (Price 1974a). This soil is a reddish-brown clay which, when wet, is strongly adherent to the skin which also holds true in the present study area. It is suggested that absorption of these irritant soils through the bare feet is responsible for the irreversible damage to the lymphatic channels resulting in elephantiasis. The present study also supports the hypothesis that "high-altitude" elephantiasis of the lower legs in East Africa is a geochemical disease (Price, 1976b; Davey and Newport, 2007; Kloos et al., 1992; and Onapa et al., 2001).

In the present study, high frequency of elephantiasis was observed in age groups (21 – 60) of both sexes. This agrees with a recent comprehensive study undertaken in Wolaitta zone, SNNP region of Ethiopia by Desta et al. (2003) in which 64% of population being in the economically productive age group as the lower legs are progressively involved during the course of several months to years. This may also support the idea of Kloos et al. (1992) that states the prevalence of non-filarial elephantiasis increases with age. This indicates that as age increases, the duration of exposurity to the work area soil-born etiology increases. On other hands, significant differences were observed among the age groups with elephantiasis more within females than males. In this condition, it also observed that males with elephantiasis were stay longer than females suggesting that males can live withstanding the effect of the disease in the given area (Table 2). The reason may be related to the lowering of immunity in females, for instance, during pregnancy.

Social stigma towards people with elephantiasis is pronounced in the study area, leading to exclusion from school, religious and other community gatherings, and a bar on marriage into non-affected families with similar fashion (Davey and Newport, 2007). Further more, in this study we have observed even some of study participants hide themselves in their kitchen or leave the family house when guests go to their or their family house, respectively. This was observed during the house-to-house survey and obtained from the nearby residents (personal observations).

The public health and socioeconomic importance of elephantiasis in the study area is indicated by its debilitating effects, which prevents more severely affected persons from day to day activities including farming and extensive walking (Fig. 4). Consequently, the affected people become dependent on their families or relatives in particular and on the country in general, or become beggars similar to elsewhere report (Price, 1974b).

According to the respondents, the illness is onset randomly within a month, and during the cold weather condition. The epidermis of the skin is peeled off from the upper and lower face of their foot or feet exposing the next layer of the skin to the secondary infection that probably caused by microbes when illness onsets and the swelling of local glands and mostly the gonad glands also occurs at this time. Disease is also onset during walking long distance, which is consistent with clinical history stated in Price (1974b). Not all individuals that are living within the same area of work were affected by this disease and our finding of a familial predisposition to non-filarial elephantiasis corroborates that of Price (1972) and Davey (2007); however, this indication needs further genetical analysis in the area and other endemic areas as well.

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Among the participants no one is having worn shoes regularly since childhood. All the affected subjects had been barefooted until the disease onset. Most of them were still without shoes even after the disease onset. However, among those who used shoes after the disease onset about 30.90% of the total has got significant change in size and relief as they respond. In this case the change observed is significantly important ($p < 0.05$). This

indicates footwear may be important to counteract to the etiology of the elephantiasis in the present study area.

In agreement with the Kloos et al. (1992) findings, younger said that they first noticed the appearance signs and symptoms in their teenage or else in adulthood although older affected persons could not remember during what specific year signs and symptoms first appeared. Further more, in contrast to, the prevalence rates were generally higher in males than in females implying sex difference due to occupationally linked trauma to the feet and the cumulative effect of long-term exposure to the soil of the area during farming (Kloos, et al., 1992) in this study, in which the rates were almost equal in both males and females, indicating no sex differences ($p > 0.05$). Indirectly this may show us that both sexes are involving in same occupation or else.

6. Conclusion

The negative results of the parasitological diagnosis and serological diagnosis; the absence of hydrocele or scrotal elephantiasis, the limitation of swelling to the lower leg (s) or feet of the affected people; the reduction of the elephantiod legs of some participants after shoe-wearing and its appearance among the same family; and the altitude range (1320 – 1640m a.s.l.) of the study area led us to conclude that the elephantiasis observed in the Ganbela and Tullu Eteya kebeles is not caused by the filarial parasites but rather by soil-borne minerals. Thus, our study suggests that endemic non-filarial elephantiasis (podoconiosis) is present in Midakegn district.

7. Recommendations

Non- filarial elephantiasis continued to be a major public health problem in tropical areas of the world as poverty is expanding with relation to human population increment and weather condition changes resulting in the formation of irritant soils from volcanic rocks. It is advisable to map out the areas where this disease is endemic to afford the victims and others with footwear to combat the problem for the next generation.

The community should be emphasized by the governmental health policy makers of the country in general and by the Midakegn district community health planners in particular. Because, the disorder now occurs wherever the irritant soils coexist with high altitudes, high rainfall, and extremely low income, predominantly where, subsistence farmers cannot afford shoes and socks. Therefore, health policy makers should give priority while they are planning on the community based prevention of diseases.

In particular, study must be undertaken to map out how much this disease affect people as a public health problem in the whole kebeles where the disease is suspected in the district so that the concerned body gets information to overcome the problem of not only heath but also of economy. Therefore, assessment of overall picture of the disease should be conducted in the district in addition to the economical impact analysis of the disease in the area as it is affecting economically productive age groups

Health education should also be given to the population of the district in general and to the study subjects in particular to save their next generation explaining that the non-filarial elephantiasis can be prevented easily if economically affordable. For instance, wearing shoes is very important for the control of non-filarial elephantiasis in addition to environmental rehabilitations and resettlements of the residents of the area.

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8. Appendices

1. Consent form

Code No _____

Name of study participant _____Age_____Sex_____

Physician Name _____ Health center_____

I have been informed about the researcher, Geleta Geshere, who is Ms Student of the Addis Ababa University and the study that planned to identify the causative agent of elephantiasis in Midakegn district under the title entitled "***Identification of Etiology of Elephantiasis in Midakegn district, Western Shoa Zone, Oromiya region*"** that will help in identifying the causative agent and distribution of elephantiasis in the area. This study could also inform the district health office in particular and the regional and federal health bureau to combat the impacts of the disease as it is a public health problem in the area.

For the study, I have been requested to give a drop of blood from my finger to the investigators. They told me that experienced health professionals according to the established aseptic procedure will collect the blood on the glass slide by finger prick using disposable lancet and in vacuum tube by venous puncture using disposable syringe needle. I have been informed that if positive result is observed treatment will be given me from the health centre of the district. Based on this, I have agreed to participate in the study/the examination. The investigator also informed me that all the laboratory results would be kept confidential.

I have been given enough time to think over before I signed this informed consent. Therefore, with full understanding of the situation I gave my informed consent and participate at my will in the course of the study.

Name (participant) _____ signature _____ Date _____

Name (investigator) _____ signature _____Date_____

Name (witness) _____ signature _____Date _____

2. Pre-sample collection population recording worksheet (Format)

No.	Name of participant	Age	Sex	Occupation	No. Family	Kebele	When illness or the disease set on (season)	Duration of onset (length in year)	Have you got treatment	Remarks

3. Questionnaire

The aim of this study is to find out the etiology of elephantiasis, the public health problem in lower altitude ecological zone in Midakegn district particularly in two kebeles, namely Tullu Eteya and Ganbela so that the district's health centre can get general information to interfere the transmission of the disease or to prevent the disease. To achieve this goal in addition to the parasitological, immunological and clinical examinations oral interview is required to get general background of the etiology of this disease in the community.

Please, the participant of this study, give appropriate answer to each interview questions asked by interviewer so that necessary information can be obtained. The information you will provide kept confidential.

1. Name _____ Registration No. _____
2. When this disease began you? _____
3. Have you used bed net before or are you using it now? _____
4. Where do you work? Farming, traditional/ local irrigation, or the combination
   If you are farmer, tell us the local name of the soil type on which you are working
   _____
   If you are using the traditional irrigation, tell us which river is used for irrigation
   _____
   If the combination _____
   Other works (shema, pottery, or blacksmith) _____
5. Have you used shoes in your life time? _____
   If you have used shoes, tell us when you began before or after the disease set on _____Do you wear shoes always? _____
6. Do the size of the swelling/ elephantiasis of your leg increase or decrease after you have used shoes? _____
7. Do you get relief after using shoes? _____
8. Do your families or relatives have this disease?_____
9. What do you think about the cause of this disease? _____
10. Do you have scrotal swelling (for males only)? _____

Declaration

I, the undersigned, declare that this thesis is my original work; has not been presented for any other purpose in any other university; and that all sources of materials used for the thesis have been correctly acknowledged.

Name: ***Geleta Geshere Oli***

Plase:- Addis Ababa University

Signature _____

Date _____

This thesis has been submitted for examination with our approval as

Advisor: Beyene Petros, Prof.

Signature _____

Date _____