2. REVIEW OF LITERATURE

During the past decade, health has achieved unprecedented prominence as a key driver of socioeconomic progress, and more resources than ever are being invested in health. Yet poverty continues to contribute to poor health, and poor health anchors large populations in poverty.

Lymphatic filariasis caused by threadlike worms, living in the lymphatic system, are *Wuchereria bancrofti*, *Brugia malayi* and *B. timori* belong to Class: Chromadorea, Order: Spirurida, Super Family: Filarioidea and Family: Onchocercidae.

2.1 History

It was well documented in Susrutha Samhita, the epic treatise in surgery, written by renowned surgeon Susrutha in 6th century B.C. The other proof from Indian origin about the disease was the description of signs and symptoms of filariasis in “Madhava Nidhan” in 7th Century A.D. by Madhava Karan. Lymphatic filariasis has been known to occur in the Nile region, and ancient artifacts suggest that the disease may have been present as early as 2000BC. A statue of Pharaoh Mentuhotep II depicts swollen limbs, a characteristic of elephantiasis, which is a symptom of heavy lymphatic filariasis infection. Artifacts from the Nok civilization in West Africa may show scrotal swelling, another characteristic of elephantiasis. The Nok artifacts date much later than the Egyptian artifacts, from about 500AD.

The first written account of lymphatic filariasis comes from the ancient Greek and Roman civilizations. In these civilizations, writers were even able to differentiate between the similar symptoms of leprosy and lymphatic filariasis, describing leprosy as "elephantiasis graecorum" and lymphatic filariasis as "elephantiasis arabum."

Discovery of Symptoms (1588-1592)

The first reliable documentation of lymphatic filariasis symptoms did not occur until an exploration of Goa between 1588 and 1592. During this trip, Jan Huygen Linschoten wrote that inhabitants were "all born with one of their legs and one foot from the knee downwards as thick as an elephant’s leg." Although
this was the first account of lymphatic filariasis symptoms, more documentation was made in parts of Africa and Asia soon after.

In 1849, William Prout became the first to document a condition common to lymphatic filariasis called chyluria. This occurs due to the passage of lymph in the urine, so it appears milky. Such a description was made in Prout's book entitled “On the Nature and Treatment of Stomach and Renal Diseases”.

**Discovery of Microfilariae (1863 and 1866)**

In 1863, French surgeon Jean-Nicolas Demarquay became the first to record the observation of microfilariae in fluid extracted from a hydrocoele (another common symptom of lymphatic filariasis). Three years later, Otto Henry Wucherer discovered microfilariae in urine in Brazil. However, the connection between these two discoveries was not made until Timothy Lewis noted the occurrence of microfilariae in both blood and urine. Lewis was also the first to make the association between the microfilariae and elephantiasis.

**Discovery of the Adult Worm (1876)**

Soon after the discovery of microfilariae, the adult worm was documented by Joseph Bancroft. The observed species was later named after Bancroft, and we now recognize it as *W. bancrofti*.

**Discovery of the Life Cycle (1877)**

Perhaps the most important discovery related to lymphatic filariasis was that made by Patrick Manson in 1877. Manson was the first to look for an intermediate host for lymphatic filariasis microfilariae. In 1877, he was finally able to pinpoint the microfilariae in mosquitoes. This discovery was later applied to other tropical diseases such as malaria, and was the first discovery of an arthropod as a vector. However, Manson incorrectly hypothesized that the transmission occurred when the mosquito deposited the filaria in water that then infected humans through ingestion of contaminated water or direct skin penetration.

**Discovery of Transmission (1900)**

In 1900, George Carmichael Low discovered microfilariae in the proboscis of mosquitoes, and finally pinpointed the true mechanism of transmission. Due
to this discovery, we now know that transmission is due to an infective bite from a mosquito vector.

2.2 Causative agent

_Wuchereria bancrofti_

_W. bancrofti_ is the most well-documented and widespread cause of lymphatic filariasis. It is more common to find elephantiasis in patients affected with _W. bancrofti_ than those affected with the Brugian filariasis. Brugian filariasis also does not characteristically include symptoms associated with the genitalia or chyluria, while Bancroftian filariasis often expresses these symptoms in heavily infected patients (John et al., 2006).

The morphology of _W. bancrofti_ is the most significant differentiation from other species. The microfilariae, or larval stage of _W. bancrofti_, are sheathed, and range from approximately 245 to 300 μm. It can take several months for the microfilariae to sexually mature, and in the adult stage they can live for several years. As adults, the males range from 2.5 to 4 cm, and the females range from 5 to 10 cm. As a roundworm, the shape of the _W. bancrofti_ name matches its descriptive classification. One end of the round body is blunt, while the other is pointed. Nuclei do not appear at the end of the tail, which is a major difference from other microfilariae. Both Bancroftian and Brugian filariae lack a digestive system, instead they absorb nutrients from their hosts (John et al., 2006).

_Brugia malayi_

The distribution of _B. malayi_ is very similar to that of _W. bancrofti_. However, cases are concentrated in Asia, including South China, India, Indonesia, Thailand, Vietnam, Malaysia, the Philippines, and South Korea. Other differences between _B. malayi_ and _W. bancrofti_ is the vector and reservoir. While _W. bancrofti_ is transmitted mainly by _Anopheles_, _B. malayi_ is transmitted by _Mansonella_ mosquitoes. Since these mosquitoes feed primarily during the day, _B. malayi_ microfilaria can be found in the blood during the day, while microfilaria of _W. bancrofti_ is found at high levels at night. The time variation in microfilarial levels is known as periodicity. Additionally, _W. bancrofti_ has no known animal reservoir, while _B. malayi_ has been found in Macaques, leaf monkeys, cats and
civet cats. In Indonesia, human cases have been transmitted from animals, which pose a particular challenge to the control of *B. malayi*.

The morphology, like that of *W. bancrofti*, is the most reliable way to differentiate species type. Generally, microfilariae range from 200 to 275 μm and adult-female worms average about 3.5 to 6 cm long while male are around 1.5 to 2.1 cm. Microfilariae of *B. malayi* are sheathed like *W. bancrofti*, and have a very similar shape. However, the nuclei extends nearly to the tip of the tail, a characteristic not shared with *W. bancrofti* (John *et al.*, 2006).

**Brugia timori**

*B. timori* is the least common, and therefore least studied species of filaria known to cause lymphatic filariasis. This species was reported on the island of Timor in 1964, and has since been found in other islands in Indonesia. In regards to vectors, periodicity and reservoirs, *B. timori* is more similar to *W. bancrofti* than to *B. malayi*. Transmission of *B. timori* is by the *Anopheles barbirostris*, a vector that feeds at night. As a result, high levels of *B. timori* microfilariae are found in the blood at night. *B. timori* also has no known animal reservoir.

In regards to symptoms and morphology, *B. timori* resembles *B. malayi* more than *W. bancrofti*. Like *B. malayi*, symptoms associated with *B. timori* are similar to *W. bancrofti*, with elephantiasis only expressed in the lower part of the limbs. Microfilaria of both *B. timori* and *B. malayi* have nuclei that extends to the tip of the tail. However, at approximately 310 μm, *B. timori* microfilaria are slightly larger than that of *B. malayi* (John *et al.*, 2006).

### 2.3 Life cycle

Like other filarial nematodes, *B. malayi* develops through four larval stages into an adult male or female (Fig. 2.1), entirely within one of two host species—a mosquito vector (*Culex, Aedes*, and *Anopheles*) and humans (or rodent in case of experimental filariasis).

*B. malayi* has a two phase life cycle, passaging between the human definitive host and a mosquito host. In the human host, adult males and females reside within the afferent lymphatic vessels just upstream from major lymph node clusters and viviparously release larvae into the bloodstream. Microfilariae are developmentally arrested until they are taken up in a blood meal by the
female mosquito vector host. In the mosquito, they resume development, escape from the peritrophic membrane and the gut, and migrate to invade the thoracic flight muscles, where they grow until again arresting as third stage larvae (L₃). After ~7 days, the infective L₃, migrate to the mouthparts where they are introduced into the vertebrate host during the next blood feeding episode. The L₃ resume growth and development, and over the next 3-4 weeks moult twice to become adults, and migrate to the lymphatic system. Microfilarial release occurs after ~100 days, and the 8 cm long adult females can live for 8 years.

Fig. 2.1: The lifecycle of *Brugia malayi*. Vertices represent molts and edges represent lifecycle stages (adopted from Gedin et al., 2007).

Both *W. bancrofti* and *B. malayi* mf show periodicity during 24 hrs cycles. They reside in pulmonary capillaries and a large proportion of this population escape into the peripheral blood for a brief period during night or day depending upon the species. *W. bancrofti* shows nocturnally periodic manner having a peak of mf density in the peripheral blood between 12 night and 2:00 am. In sub-periodic strains mf circulate throughout the 24 hrs with low density but their density increases during night or day depending upon the species.
2.4 Epidemiology

Global Distribution

Lymphatic filariasis is endemic in 83 countries (WHO, 2006b) mainly in the tropics, both north and south (Fig. 2.2). India, Indonesia, Nigeria and Bangladesh account for nearly 70% of lymphatic filariasis cases. Other regions include Central Africa, the Nile delta, Pakistan, Sri Lanka, Burma, Thailand, Malaysia, Southern China, the Pacific Islands, Haiti, the Dominican Republic, Guyana, Surinam, French Guiana, and Brazil (John et al., 2006).

Fig. 2.2: Lymphatic filariasis Endemic countries and territories (Source: The Carter Center)

Economic burden of disease and risk

The 'at-risk' population for contraction of lymphatic filariasis includes 1.2 billion people. Currently, more than 120 million people are affected by lymphatic filariasis, including 25 million men who suffer from the genital swellings associated with the disease and 15 million people who suffer from severe lymphodema or elephantiasis of the leg.

The economic burden of lymphatic filariasis is tremendous. Patients who are heavily infected with lymphatic filariasis have a high risk of developing chronic symptoms, including lymphodema and elephantiasis. Such symptoms can result in a decrease in productivity, as they can lead to life-threatening infections if not properly cared for, as well as mobility and functionality problems. In India alone, it is estimated that the annual economic loss due to
lymphatic filariosis is $1 billion USD (The Carter Center, 2006). In nations that are endemic, the economic losses are often not calculated, but likely significant.

The perception upon disease by various agencies are usually based on the market value of any investment for the diseases and possible returns. Some regions of the world that can afford medicine mainly consisting of developed nations such as the U.S. and Europe generally represent the market of individuals who can pay for therapies. In contrast there are so many more types of diseases prevailing in the developing countries and causing suffering to the humanity to a much higher extent (Fig. 2.3).

![DALY burden per disease in Developed countries](image1) ![DALY burden per disease in Developing countries](image2)

**Fig. 2.3 (A).** DALY burden in regions of the world that can afford medicine (mainly developed nations such as the U.S. and Europe. (B). Comparative burden of above with developing countries (bars added adjacent to the developed country data.

In the 21st century, health is a shared responsibility, involving equitable access to essential care and collective defence against trans-national threats. Non profitable agencies like World Health Organization (WHO) acts as the directing and coordinating authority for health within the United Nations system. It operates on the principle that health development is directed by the ethical principle of equity: Access to life-saving or health-promoting interventions should not be denied for unfair reasons, including those with economic or social roots. It is responsible for providing leadership on global health matters, shaping the health research agenda, setting norms and standards, articulating evidence-based policy options, providing technical support to countries and monitoring and assessing health trends. Committed to these view points it collects epidemiological data related to various health aspects.
Table 2.1A: Cause and gender wise distribution of Global burden of diseases in terms of Disability adjusted life years (DALY) and Years lost to Disability (YLD).

<table>
<thead>
<tr>
<th>Population</th>
<th>DALY (World)</th>
<th>DALY (India)</th>
<th>YLD (World)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
<td>Total</td>
</tr>
<tr>
<td>A. Infectious and parasitic diseases</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Tuberculosis</td>
<td>159,74</td>
<td>142,403</td>
<td>302,144</td>
</tr>
<tr>
<td>2. Tuberculosis</td>
<td>21,65</td>
<td>12,558</td>
<td>34,217</td>
</tr>
<tr>
<td>3. STDs excluding HIV</td>
<td>5,558</td>
<td>6,866</td>
<td>10,425</td>
</tr>
<tr>
<td>4. Diarrhoeal diseases</td>
<td>30,905</td>
<td>34,872</td>
<td>72,717</td>
</tr>
<tr>
<td>5. Childhood-cluster diseases</td>
<td>16,221</td>
<td>14,005</td>
<td>30,226</td>
</tr>
<tr>
<td>6. Meningitis</td>
<td>5,891</td>
<td>5,536</td>
<td>11,424</td>
</tr>
<tr>
<td>7. Hepatitis</td>
<td>2,090</td>
<td>932</td>
<td>3,023</td>
</tr>
<tr>
<td>8. Malaria</td>
<td>17,340</td>
<td>16,636</td>
<td>33,976</td>
</tr>
<tr>
<td>9. Tropical-cluster diseases</td>
<td>8,264</td>
<td>3,850</td>
<td>12,113</td>
</tr>
<tr>
<td>10. Leprosy</td>
<td>116</td>
<td>78</td>
<td>194</td>
</tr>
<tr>
<td>11. Dengue</td>
<td>336</td>
<td>334</td>
<td>670</td>
</tr>
<tr>
<td>13. Trachoma</td>
<td>338</td>
<td>997</td>
<td>1,334</td>
</tr>
<tr>
<td>14. Intestinal nematode infections</td>
<td>2,052</td>
<td>1,961</td>
<td>4,013</td>
</tr>
<tr>
<td>15. Respiratory infections</td>
<td>51,266</td>
<td>46,520</td>
<td>97,786</td>
</tr>
<tr>
<td>16. Other infectious diseases</td>
<td>28,558</td>
<td>15,074</td>
<td>43,842</td>
</tr>
<tr>
<td>B. Noncommunicable diseases</td>
<td>378,693</td>
<td>355,959</td>
<td>731,652</td>
</tr>
<tr>
<td>1. Malignant neoplasms</td>
<td>41,893</td>
<td>35,919</td>
<td>77,812</td>
</tr>
<tr>
<td>2. Other neoplasms</td>
<td>1,016</td>
<td>937</td>
<td>1,953</td>
</tr>
<tr>
<td>3. Diabetes mellitus</td>
<td>9,046</td>
<td>10,659</td>
<td>19,705</td>
</tr>
<tr>
<td>4. Endocrine disorders</td>
<td>4,793</td>
<td>5,653</td>
<td>10,446</td>
</tr>
<tr>
<td>5. Neuropsychiatric conditions</td>
<td>98,328</td>
<td>100,952</td>
<td>199,280</td>
</tr>
<tr>
<td>6. Sense organ diseases</td>
<td>41,843</td>
<td>45,040</td>
<td>86,883</td>
</tr>
<tr>
<td>7. Cardiovascular diseases</td>
<td>82,894</td>
<td>68,483</td>
<td>151,377</td>
</tr>
<tr>
<td>9. Digestive diseases</td>
<td>24,657</td>
<td>17,841</td>
<td>42,498</td>
</tr>
<tr>
<td>10. Genitourinary diseases</td>
<td>8,735</td>
<td>6,019</td>
<td>14,754</td>
</tr>
<tr>
<td>11. Skin diseases</td>
<td>1,936</td>
<td>1,943</td>
<td>3,879</td>
</tr>
<tr>
<td>12. Musculoskeletal diseases</td>
<td>13,604</td>
<td>17,265</td>
<td>30,869</td>
</tr>
<tr>
<td>13. Congenital anomalies</td>
<td>12,853</td>
<td>12,427</td>
<td>25,280</td>
</tr>
<tr>
<td>14. Oral conditions</td>
<td>3,878</td>
<td>3,997</td>
<td>7,875</td>
</tr>
<tr>
<td>C. Maternal, perinatal &amp; nutritional conditions</td>
<td>83,069</td>
<td>120,995</td>
<td>204,062</td>
</tr>
<tr>
<td>D. Injuries</td>
<td>123,366</td>
<td>64,249</td>
<td>187,614</td>
</tr>
<tr>
<td>Total Burden</td>
<td>744,869</td>
<td>680,606</td>
<td>1,425,472</td>
</tr>
</tbody>
</table>
Table 2.1B: Contribution of Lymphatic filariasis (grouped under Tropical cluster diseases) in Global burden of diseases in terms of DALY and YLD. Source: WHO estimates for 2004; All figures in thousands.

<table>
<thead>
<tr>
<th>Tropical cluster diseases</th>
<th>DALY (World)</th>
<th>DALY (India)</th>
<th>YLD (World)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
<td>Total</td>
</tr>
<tr>
<td>1. Trypanosomiasis</td>
<td>1,041</td>
<td>631</td>
<td>1,673</td>
</tr>
<tr>
<td>2. Chagas disease</td>
<td>231</td>
<td>199</td>
<td>430</td>
</tr>
<tr>
<td>3. Schistosomiasis</td>
<td>1,021</td>
<td>686</td>
<td>1,707</td>
</tr>
<tr>
<td>4. Leishmaniasis</td>
<td>1,227</td>
<td>748</td>
<td>1,974</td>
</tr>
<tr>
<td>5. Lymphatic filariasis</td>
<td>4,521</td>
<td>1,420</td>
<td>5,941</td>
</tr>
<tr>
<td>6. Onchocerciasis</td>
<td>223</td>
<td>166</td>
<td>389</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>8,264</td>
<td>3,850</td>
<td>12,113</td>
</tr>
</tbody>
</table>

Disability adjusted Life years (DALY)

The number of deaths is not a very informative indicator of ill health. Better is the loss of healthy life entailed by injury, disease, and premature death.

The DALY or burden of disease statistics enables estimates to be made of the proportion of morbidity and premature mortality that can be attributed to specific risk factors. From the global burden of disease (GBD), DALY is one such measure becoming common in international comparisons (Murray and Lopez, 1996). The DALY basically indicates the amount of healthy life expectancy lost because of a disease or risk factor, including both mortality and morbidity (Table 2.1).

Hold in endemic countries

Nations found to be endemic tend to be tropical or subtropical due to the optimal habitat for the vectors of lymphatic filariasis. Ambient humidity is also necessary for the survival of the infective larva stage of the filaria parasite. Populations at high risk for contracting or developing a lymphatic filariasis infection are primarily poor, and a majority of the cases are concentrated in rural areas. Lymphatic filariasis is often associated with areas that have poor sanitation and housing quality (GAELF, 2006). Poorer, rural communities are also typically built around optimal environments for vectors, including marshes or rivers, and tend to lack the resources or capabilities to control for vectors, and as a result, transmission is high.

Although there is an established high prevalence of transmission in rural areas of endemic areas, approximately 6% of urban cases of lymphatic filariasis
were results of urban transmission. This percentage is high enough to confirm transmission of lymphatic filariasis in urban areas (Terranella et al., 2006).

2.5 Symptoms and pathology

Although most of the symptoms of Brugian filariasis are identical to Bancroftian filariasis, there are some differences in clinical presentation. First, Brugian filariasis tends to have a higher occurrence of ulcerated nodules, and rarely involve genital swelling or chyluria. In addition, elephantiasis is experienced almost explicitly in the lower part of the limbs, below the knee or elbow (John et al., 2006).

The nematode parasite alone is not responsible for all the symptoms; rather the host immune response against the parasite is the major one. The most severe one is the damage to the lymphatic vessels which is mediated by the immune responses to the adult worms living in them. The characteristics of these responses are inflammation (lymphangitis) of the affected area, generalized malaise and fever. Repeated episodes of lymphangitis or acute manifestations lead to the formation of fibrous and calcified tissues in and around the lymphatic vessels. These results in chronic manifestations characterized by grotesque deformities and are usually irreversible.

2.5.1 Sequential development of filariasis

This may be classified into the following stages:

(i) Pre patent period (biological incubation period)

It is the time between entry of infection stage larvae to the development of adult worms and appearance of mf in the circulating blood of the host. It has been estimated to require a year or more for W. bancrofti. However, the B. malayi takes 3 - 4 months to develop in the definitive host.

(ii) The patent (symptom-less) period

This stage is characterized by the presence of mf in the peripheral blood but without any clinical manifestations of filariasis. This is the most important group that serves as the secondary carrier of infection. A considerable proportion of population remain microfilaraemic and asymptomatic for years together and in some instances for whole life. However some individuals become amicrofilaraemic while other may progress more rapidly to the acute and chronic
stages. Most of the asymptomatic cases have lymphatic abnormalities as detected by lymphoscintigraphy and also renal abnormalities, which is evidenced by hematuria and/or proteinuria (Dreyer et al., 1992; Freedman et al., 1999).

(iii) The acute or allergic stage

The acute clinical manifestations of filariasis are characterized by episodic attacks of adenolymphangitis (ADL) with constitutional symptoms like fever, chills, malaise, nausea, headache and vomiting (WHO, 1998b). In bancroftian filariasis the ADL attacks occur usually in the limbs, groin and the male genitalia during the acute stage, leading to funiculitis, epididymitis or orchitis. The repeated attacks of ADL precede the development of chronic lymphatic pathology of filariasis and these often continue for many years (Pani et al., 1994). ADL lasts usually for 3 - 5 days but sometimes may stay upto 15 days. Lymphoedema is frequently present during the episodes and usually subsides after acute stage. However sometimes it does not subside and lead to chronic changes (Dissanayake et al., 1984).

(iv) Chronic manifestations

The most conspicuous feature of clinical symptoms caused as a result of filarial infection is noted in the chronic stage. This occurs due to blockage of lymphatics. The major chronic signs are hydrocele, chyluria, lymphoedema and elephantiasis, which may differ in occurrence from one area to another. More serious are the blockage of the abdominal or thoracic lymph vessels, which eventually cause chyluria or hematochyluria. This stage is often incurable.

2.5.2 Categories filarial subjects

In an endemic population almost all individuals are exposed to the mosquito bites, and so an equal probability prevails for individuals to be exposed to inoculation of L3. However all individuals do not develop into similar state of infection. Following infection with L3 there is usually a period of vigorous immune responses to the invading larvae. If the larvae are not cleared from the body during this period then various pathologies associated with filarial infection can develop. The most pronounced of these is the damage to the lymphatic vessels, which is mediated by the immune responses to the adult worms living in them. These immune responses are characterized by inflammation (lymphangitis) of the affected area, generalized malaise and fever. Irrespective of the infection
exposure dose and course of development of infection, different individuals respond differently. This is an important feature of human lymphatic filariasis that, not all hosts develop microfilaremia (Lawrence and Devaney, 2001). So there exist groups of individuals ranging from microfilariae negative, to infection positive but without symptoms, and to manifestation of chronic disease in the form of elephantiasis.

(a) Mf carriers
Most of the people living in endemic area show mf in their peripheral blood and remain as such throughout life. These are called as mf carriers. They lack recognizable clinical manifestations in their entire life. Asymptomatic mf carriers do not always present with overt clinical manifestations, but lymphatic pathology in the form of dilation, kinking, collateral formation, etc., are common. They are involved in spreading of disease.

(b) Symptomatic
These are the individuals in whom the disease has proceeded to the acute stage. The most pronounced of these is the damage to the lymphatic vessels, which is mediated by the immune responses to the adult worms living in them. These acute immune responses are characterized by Lymphangitis (inflammation of the affected area), generalized malaise and fever. Later the major chronic signs appear as hydrocele, chyluria, lymphoedema and elephantiasis. More serious are the blockage of the abdominal or thoracic lymph vessels, which eventually cause chyluria or hematochyluria and is often incurable. Symptoms due B. malayi infection are largely same as that due to W. bancrofti. However fever associated with lymphangitis in patients from endemic areas of B. malayi is more common than in those of W. bancrofti. Lymphoedema and elephantiasis of legs and arms are common in both but the absence of involvement of the genito-urinary organs is a characteristic feature of B. malayi filariasis. Though mostly limbs are affected, the upper extremities are much less frequently affected than the legs.

(c) Asymptomatic
In most of the endemic areas a proportion of the population remained mf negative and is devoid of symptoms of the disease despite life long exposure to infection. It is possibly heterogeneous group consisting of “Truly Immune” cases
apart from those with prepatent or sub clinical or unisexual infections of parasite.

(d) Other forms of filariasis

i. Occult filariasis

The term occult filariasis is commonly used to designate filarial infections in which classical clinical manifestations are not present and mf are not found in the peripheral blood although they may be seen in tissues. However, it has now been shown that in some cases with occult filariasis, mf may actually be found after more careful blood examination despite their low density. Occult filariasis is believed to result from a hypersensitivity reaction to filarial antigens derived from microfilariae. Only a very small proportion of individuals in a community where filariasis is endemic develops occult forms of the disease. Occult filariasis with adult *W. bancrofti* in the anterior chamber of the eye has also been reported (Arora and Das, 1990; Nanavaty, 2001; Rao, 2008).

Other manifestation such as mono/polyarthritis, hematuria, glomerulopathies (glomerulonephritis), endomyocardial fibrosis, oligoarticular filarial arthritis, and filarial pseudo-rheumatism are also well recognized.

ii. Tropical Pulmonary Eosinophilia

Tropical pulmonary eosinophilia (TPE) is an occult manifestation of filariasis (Narayanan *et al.*, 2003). Its main clinical manifestations are: nocturnal paroxysms of asthmatic symptoms (severe cough and wheezing, specially at night), frequent weight loss and fatigue but with minimal or no fever, restrictive or obstructive lung abnormalities, abnormal chest radiographs that frequently show diffuse mottled pulmonary interstitial infiltrate. The peripheral blood eosinophilia increases to very high extent (> 3000 cell/μl), extreme elevation of antifilarial immunoglobulins (IgE and IgG). Dramatic clinical improvement in response to specific anti-filarial chemotherapy with (DEC) has been observed (McCarthy *et al.*, 1995). The syndrome is characterized by nocturnal paroxysms of asthmatic symptoms, persistent eosinophilia and later by chronic interstitial lung disease (Piessens and da Silva, 1982).
2.5.3 Pathogenesis

Host immune and inflammatory responses to infections that are poorly controlled may underlie various chronic diseases. Immune responses, both adaptive and innate, can lead to the development of inflammation. These responses may result in the production of antibodies of specific isotype, immune complex deposition, antibodies against cellular targets, or T cell-mediated responses. Inflammatory responses typically help to eliminate offending parasites. But those same inflammatory responses may also damage surrounding healthy tissues. If the parasite persists or if the normal signaling pathways that down-regulate the inflammatory response are disordered, then an ongoing inflammatory response may develop. The tissue damage resulting from this response may be the dominant portion of the clinical disease (Pincus, 2005).

The pathology of lymphatic filariasis has three components: (1) parasitological (including elements of rate of infection and parasite mortality); (2) immunological (according to selection of immune mechanisms and target antigens); and (3) microbiological (secondary opportunistic infections that exploit damage induced by the presence of filariae). Dissection of these factors is complicated, especially as any group of human patients will include persons at different stages of disease development, varying from those who still retain large parasite numbers to those whose lesions have outlived the filarial worm population. Moreover, it is possible that elephantiasis can result from either parasitological or immunological causes, and the search for a unifying character may be fruitless. The parasitological component of pathogenesis includes direct effects such as lymphatic dilation induced by adult worms which reduces flow and leads to incompetence. There may also be dynamic effects related to the pattern of infection; cats infected repeatedly with small doses show a higher rate of lymphatic pathology than do those given single, large inoculums. Immunologically, elephantiasis cases show high T-cell proliferative responses and high IgE and IgG1, 2 and 3 to filarial antigens. However, a significant minority of these patients have a microfilaraemic-like phenotype, bearing hyporesponsive T cells with high IgG4. This finding casts doubt on either IgE or inflammatory T cells being the initiating factor in lymphatic lesions. Furthermore, many healthy endemic normals can be found with IgE and T-cell responses comparable to those seen in elephantiasis. One suggestion has been that
normals avoid pathology by killing incoming parasites promptly before entry into the lymphatics. A more-interesting lead is the high levels of IgGl, 2 and 3 seen in elephantiasis relative to both microfilaraemics and normals; it should also be noted that these isotypes are elevated in elephantiasis patients irrespective of their T-cell or IgE responses (Maizels et al., 1995).

2.6 Immune response to filaria infection

Due to long association, immune response of host to parasite in filariasis infection results a wide range of clinical and pathological manifestations represented by asymptomatic carriers of mf at one pole to symptomatic cases of filariasis with or without circulating mf and extreme cases of elephantiasis to other pole.

2.6.1 Molecular approaches to understand parasite survival

To succeed in infection, parasites must have ways to reach the host, penetrate its tissues and escape its defense systems. As they are not fatal, most helminth parasites remain viable within their host for many years, exerting a strong influence over the host immune function. Many of these functions are performed by products that are released from the parasite (Moreno and Geary, 2008). Studies with mouse models confirm that, as in humans, both innate and adaptive arms of host immunity are targeted by filarial parasites (Hoerauf et al., 2005; MacDonald et al., 1998; Taylor et al., 2005).

Macrophages

Macrophages play crucial roles in the immune response, as they can initiate, modulate and also be final effector cells during immune responses to infections. Macrophages are derived from myeloid precursor cells in bone marrow and are widely distributed in every tissue of the body. Over the past 10 years, the major support for the current concept of alternatively activated macrophages (AAMφ) comes from parasitic helminth infections. Parasitic helminths have developed complex mechanisms to evade and modulate host immunity. Given the recent advances in understanding the immunoregulatory capabilities of helminthic infections, it has been suggested that macrophages can be a target for immunomodulation. Furthermore, they become altered when a host experiences chronic exposure to helminth parasites or their by-products,
which favour the induction of AAMφ. How AAMφ participate in modulating host immunity during helminth infections and what their roles are in clearing or favouring parasite survival remains elusive (Reyes and Terrazas, 2007).

**Dendritic cells**

Innate recognition of infection in vertebrates can lead to the induction of adaptive immune responses through activation of dendritic cells (DCs). DCs are activated directly by conserved pathogen molecules and indirectly by inflammatory mediators produced by other cell types that recognise such molecules. In addition, it is likely that DCs are activated by poorly characterised cellular stress molecules and by disturbances in the internal milieu. The multiplicity of innate pathways for DC activation may have evolved to ensure that any signs of infection are detected early, before overwhelming pathogen replication. Toll-like receptor (TLR) activation following recognition of pathogens is one of the main pathways through which DCs become activated during infections (Reis e Sousa, 2004). For MHC class II-mediated peptide antigen presentation, the functionally important transitions in DC differentiation are well studied. Quiescent myeloid cells first acquire the ability to rapidly internalize exogenous antigens and cleave them into peptides as they develop into immature DCs (Moody, 2006). Subsequently, the transition from immature to mature DCs involves release of peptide-MHC complexes from endosomes to the surface, expression of costimulatory molecules and other changes, all of which are critical for controlling whether the TCR stimulation results in T cell priming or anergy (Steinman et al., 2003). Thus, two functionally important transitions in DC maturation regulate the stimulation of MHC class II-restricted CD4+ T cells (Moody, 2006). In patent cases of lymphatic filariasis antigen-specific T-cell unresponsiveness is observed with diminished IFN-γ and IL-2 production and defects in dendritic cell (DC) function. Mf interfere with monocyte-derived human DCs (mhDCs) function by altering TLR expression and interfering with both MyD88-dependent signaling and a pathway that ultimately diminishes NF-κB activity. This down-regulated NF-κB activity impairs mhDC-produced cytokines needed for full T-cell activation (Semnani et al., 2008).

Lymphatic filariasis necessitates immune dysregulation involving APC and T cell populations. In filaria-infected individuals the baseline expression of TLR is lower in B cells than uninfected ones. Filarial Ag stimulates a diminished up-
regulation of TLR in both B cells and monocytes of infected individuals. Stimulation of B cells and monocytes with TLR ligands result in decreased B cell and monocyte activation/cytokine production, indicating a state of immune tolerance. This dysregulation is associated with diminished CD4(+) T cell production of IFN-γ and IL-5. The diminished expression and function of TLR is a likely consequence of chronic antigen stimulation and is one of the underlying mechanisms of dysfunctional immune response in filariasis (Babu et al., 2005).

Activation of T cells

T cell activation is dependent upon signals delivered through the antigen-specific T cell receptor and accessory receptors on the T cell. A primary costimulatory signal is delivered through the CD28 receptor after engagement of its ligands, B7-1 (CD80) or B7-2 (CD86). Engagement of CTLA-4 (CD152) by the same B7-1 or B7-2 ligands results in attenuation of T cells responses (Carreno and Collins, 2002). T cells from infected individuals are associated with the presence of a number of markers linked to regulatory T cells (Babu et al., 2006b; Steel and Nutman, 2003). Defects in the antigen presenting cell (APC) population are also evident (Semnani and Nutman, 2004), as exemplified by reduced monocyte responsiveness to inflammatory stimuli (Sasisekhar et al., 2005).

Toll-Like Receptors

Stimulation of different TLRs induces distinct patterns of gene expression, which not only leads to the activation of innate immunity but also instructs the development of antigen-specific acquired immunity (Akira and Takeda, 2004). Activation of TLR leads to TLR response genes (TRGs), such as inducible nitric oxide synthase (NOS2), which generates microbicidal nitric oxide (NO) and cytokines such as interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), interferons (IFNs) and chemokines. TLR2 plays an essential role in filaria-induced dendritic cell activation and IFN-γ production, but does not affect filaria-induced Th2-associated responses (Daehnel et al., 2007). The innate inflammatory pathways activated by endosymbiotic Wolbachia in B. malayi and O. volvulus filaria are dependent on TLR2-TLR6 interactions and are mediated by adaptor molecules MyD88 and TIRAP/ MyD88 adaptor-like (Mal) (Hise et al., 2007).
2.7 Disease management

2.7.1 Diagnosis

LF was diagnosed clinically by blood films taken at night, as most forms of human filarial parasites have nocturnal periodicity. The limited sensitivity of blood films led to the development of concentration techniques (nucleopore filtration) or detection in larger quantities of lysed blood using a counting chamber. A DEC-based provocative test was also used in some settings if night blood films could not be done, as the treatment with DEC 'provokes' the appearance of microfilaria in the blood within 30-45 min of DEC administration, during the day (Molyneux, 2009). It can also be established following a laboratory examination revealing hypereosinophilia corresponding to the incidental finding of microfilariae (blood or skin). The visualization of the embryonic and/or adult parasite confirms the infection. For pathogenic filariasis with microfilaremiae, paradoxically, clinically positive subjects are often amicrofilaremic. In this case, the presence of antibodies and/or specific serum antigens confirms the diagnosis (Carme, 2007).

Other methods include detection of 'filaria dance sign' (Amaral et al., 1994) by ultrasonography and gamma camera imaging based observations called lymphoscintigraphy which detects structural changes like lymphatic dilatation, dermal back flow and obstruction in the oedematous limbs even in the early, clinically asymptomatic stage of the disease (McCarthy, 2000; Palumbo, 2008).

Whilst such methods are appropriate for individual diagnosis and succeeded in some settings where patients identified were treated with DEC (selective treatment), the current strategies of MDA (Ottesen, 2006) require a different approach. This applies initially to mapping distribution, which has been based on the antigen detection test known as the immuno-chromatographic test (ICT) (Weil et al., 1997) that detects circulating filarial antigen. This simple test was used to map the prevalence at the implementation unit level in several settings before the initiation of national LF control programmes (Gyapong et al., 2002; Onapa et al., 2005). The test is also recommended for assessing progress towards elimination endpoints. The alternative to the ICT for measuring antigen is the ELISA-based approach using the Og4C3 monoclonal antibody (Njenga et al., 2007). The advantage of the ICT in detecting antigen allows a more
immediate assessment of success, particularly if cohorts of children born since
the intervention began are tested as this group will be the most sensitive in
terms of exposure to infection after the initial MDA. The WHO has provided
detailed guidelines for monitoring and evaluation of LF programmes. However, it
is difficult not to utilise the gold standard microscopy approach, hence sentinel
sites are earmarked for ongoing examination by night blood films. Samples of 60
μl of blood taken between 22:00 h and 04:00 h in 500 adults provide the key
information. However, this evaluation tool does create a range of problems—
unsocial hours, extensive microscopy and reduction in sensitivity. For this
reason, other approaches to endpoint evaluation and surveillance are being
appraised. These include exposure antibodies in children (Njenga et al., 2007)
and PCR methods for xenomonitoring filarial parasites in mosquitoes (Ottesen,
2006; Ramzy et al., 2006).

It can also be established following a laboratory examination revealing
hypereosinophilia or correspond to the incidental finding of microfilariae (blood
or skin). The visualization of the embryonic and/or adult parasite confirms the
infection. For pathogenic filariasis with microfilaremiae, paradoxically, clinically
positive subjects are often amicrofilaremic. In this case, the presence of
antibodies and/or specific serum antigens confirms the diagnosis. On the
contrary, asymptomatic microfilariae carriers are common but there is no
guarantee that they will remain asymptomatic. The etiological treatment of
choice is based on a combination of ivermectin and albendazole. However,
diethylcarbamazine, which was formerly used, is still indicated (Carme, 2007).

2.7.2 Treatment and control

Diethylcarbamazine (DEC): This drug is effective against both microfilaria and
adult worms. DEC markedly lowers the blood microfilaria levels even in single
annual doses of 6 mg/kg, and this effect is sustained even after one year. Even
though DEC kills the adult worms, this effect is only observed in 50% of
patients. By ultrasonography it is shown that even single doses of DEC kill the
adult worms when they are sensitive to the drug. When they are not sensitive
even repeated doses do not show any effect on the adult parasite. This drug
does not act directly on the parasite but its action is mediated through the
immune system of the host. The earlier recommended dose of this drug was 6
mg/kg given daily for 12 days. Recent studies have shown that a single dose of
DEC 6 mg/kg is as effective as the above standard dose given for 12 days. The sustained destruction of microfilaria by this drug even in annual single doses makes it a good tool to prevent the transmission of this disease. The adverse effects produced by the drug are mostly observed in patients who have microfilaria in their blood and are due to their rapid destruction which is characterized by fever, headache, myalgia, sore throat or cough lasting for 24 to 48 hours (Andrade et al., 1995). They are usually mild and self-limiting requiring only symptomatic treatment. Direct adverse effects related to the drug are very rare. Recent trials have clearly shown that DEC has no action either in the treatment or prevention of the acute ADL attacks occurring in lymphoedema (Shenoy et al., 1999). DEC is the drug of choice in the treatment of Tropical Eosinophilia syndrome in which it should be given for longer periods of 3 to 4 weeks.

Ivermectin (IVM): This drug acts directly on the microfilaria and in single doses of 200 to 400 μg/ kg keeps the blood microfilaria counts at very low levels even after one year, such as DEC. The adverse effects noticed in microfilaraemic patients are similar to those produced by DEC but are milder due to the slower clearance of the parasitaemia. IVM has no proven action against the adult parasite or in tropical eosinophilia (Dreyer et al., 1996). IVM is the drug of choice for the treatment of onchocerciasis because of its safety and efficacy, when compared to DEC. It is also the drug of choice for the prevention of filariasis in African countries endemic for Onchocerca and Loa loa, where DEC cannot be used due to possible severe adverse reactions. IVM is also effective against human ectoparasites such as head and body lice, scabies (var hominis) and many intestinal helminths. This drug is not licensed for human use in India (Palumbo, 2008).

Albendazole (ALB): This antihelminthic drug is shown to destroy the adult filarial worms when given in doses of 400 mg twice a day for two weeks. The death of the adult worm induces severe scrotal reactions in bancroftian filariasis since this is the common site where they are lodged (Jayakody et al., 1993). ALB has no direct action against the microfilaria and does not immediately lower the microfilaria counts. When given in single dose of 400 mg in association with DEC or IVM, the destruction of microfilaria by these drugs becomes more pronounced. ALB combined with DEC or ivermectin is recommended in the global filariasis elimination programme. The strategy that appears most suitable
for the elimination of filariasis in India is the administration of a single annual dose of ALB 400 mg along with DEC 6 mg/kg of body weight. This not only prevents transmission of filariasis in the community by reducing the microfilaria levels, but also has the added benefit of clearing the intestinal helminths (Shenoy et al., 2000).

**Combination therapy:** The above drugs have also been studied for possible synergistic effects by co-administration in combinations (like ALB+ IVM, ALB+DEC, ALB+DEC+IVM, or DEC+IVM) in various populations. The studies indicate certain degree of additive protection from microfilaria or adult worms. Results with ALB added to single-drug therapy with IVM or DEC against lymphatic filariasis were inconclusive, but DEC and IVM in combination appeared to be superior to DEC or IVM alone. None of the drug combinations against lymphatic filariasis showed more adverse reactions than single-drug therapy (Olsen, 2007).

**Antiwolbachial therapy:** There is another complementary chemo-therapeutic approach that leads to a long-lasting reduction of the pathology-inducing worm stages, or to a macrofilaricidal effect. The recent discovery that depletion of Wolbachia endosymbionts by tetracycline antibiotics leads to long-lasting sterility of adult female worms in onchocerciasis and a macrofilaricidal effect in LF fulfills these requirements. The antiwolbachial chemotherapy can currently be applied in the form of a suitable doxycycline regimen for 6 weeks for the treatment of individuals, and exploited in the future for the development of new drugs suitable for mass application. In addition, first data suggest that Wolbachia may also be major mediators of lymphangiogenesis and that their depletion is associated with reduction of lymph vessel-specific vascular endothelial growth factors and reduced lymph vessel size (Hoerauf, 2006).

By far MDA is the most successful strategy toward the goal of elimination of lymphatic filariasis. What further needed is that it will be still necessary to ensure a sustained global drug pressure and an active surveillance to prevent the re-emergence of the disease. Added to this are other preventive measures that would support the elimination efforts in wider geographical areas.

Vector control was the primary tool for controlling filariasis in several part of world and is preferred because it prevents other diseases simultaneously (Burkot et al., 2007). Covering water-storage containers and improving waste-water and solid-waste treatment systems can help by reducing the amount of
standing water in which mosquitoes can lay eggs. In addition, killing eggs (ovicidal) and killing or disrupting larva (larvicidal) in bodies of stagnant water can further reduce mosquito populations.

Some recent studies have shown that with proper 'local care' of the affected limb these ADL attacks can be prevented even in case of severe lymphoedema. This 'foot-care programme' involves washing of the affected area, especially the webs of the toes and deep folds of skin, with soap and water twice a day or at least once before going to bed and wiping dry with a clean cloth to avoid moisture; clipping the nails at intervals and keeping them clean; preventing or promptly treating any local injuries or infections using antibiotic ointments (McCarthy, 2000). Regular use of properly fitting foot wear; raising the affected limb at night in order to reduce the swelling; to prevent repeated ADLs in such patients, administration of long term antibiotic therapy with oral penicillin or long acting parenteral benzathine penicillin (Palumbo, 2008).

Once lymphoedema is established there is no cure. However the following treatment offers relief and may prevent further progression of the swelling: using elastocrepe bandage or tailor made stockings while ambulant; keeping the limb elevated at night or while resting, after removing the bandage; regular exercise of the affected limb; regular light massage of the limb to stimulate the lymphatics and to promote flow of lymph towards larger patent vessels; intermittent pneumatic compression of the affected limb using single or multicell jackets; heat therapy either using wet heat or hot oven; surgical procedures such as lymph nodo-venous shunts, omentoplasty, excisional surgery, skin grafting; prolonged treatment with oral or topical coumarin or flavonoids is considered to be effective in reducing the lymphoedema (Palumbo, 2008).

**Therapeutic and vaccine targets**

The different forms of the parasite inhabit different compartments in the mammalian host. Unique set of proteins released by each form reflecting particular developmental processes and different strategies for evasion of host responses (Moreno and Geary, 2008). Other comparative analysis of proteins in secretome and adult parasite demonstrates selective release of a suite of newly identified proteins not previously suspected to be involved at the host–parasite interface, and provides important new perspectives on the biology of the filarial parasite in terms of therapeutic and vaccine targets (Hewitson et al., 2008).
Vaccine targets

Over the past few years, a number of advancement has been made towards identifying antigens molecules for vaccine development. Earlier efforts for in search of a vaccine candidate involved use of attenuated (by irradiation) third-stage larvae (L3) of *B. malayi* (Abraham et al., 1989), or killed mf (Hayashi et al., 1989). Later several individual proteins of *B. malayi* were also tested for their prophylactic activity and found different levels of protection. Abundant larval transcript-2 (BmALT-2), recombinant *Brugia malayi* transglutaminase (BmTGA), thioredoxin peroxidase (BmTPX) (Gnanasekar et al., 2004; Vanam et al., 2009) showed 35 to 70 percent activity on immunization of experimental animals. Other functional protein molecules of parasite like glutathione-S-transferase, of *Setaria cervi* (Gupta et al., 2005), *B. malayi* Super Oxide Dismutase (BmEC-SOD) that are involved in the antioxidant system are also exploited (Dabir et al., 2008) for such purpose. Several antigens of unknown biochemical function are also tried showing various degrees of protection, namely - Bm-SL3 (37-kDa) (Dabir et al., 2006), Bm mf S-7 (38kDa ) (Krithika et al., 2005), 175 kDa collagenase (from *setaria*) (Pokharel et al., 2006), filarial SXP-1 (antigen present in multiple worm stages) and r-chitinase (Wang et al., 1999).

Although immunization with recombinant antigens has been actively investigated (Fischer et al., 2003; Perbandt et al., 2005; Wu et al., 2004), the most effective protection is still obtained with irradiated larvae. This is consistent with other helminth systems as immunization with defined antigens of *Schistosoma mansoni* is less effective than with attenuated infective stages (Ganley-Leal, 2005).

Many other potentially protective antigens were identified basing on seroreactivity in various population groups in endemic areas. Filarial antigens of approximately 25, 42, 60, and 112 kDa (Nilsen et al., 1988), 63 kDa antigen (Perrine et al., 1988), 109, 102, 97 and 77; 66, 46, 35, 33, 30 and 14 kDa protein (Kharat et al., 1989), 62-kDa *Brugia malayi* antigen (Kazura et al., 1992), 120 kDa *Brugia malayi* adult antigen fraction, BmA-2 (Chenthamarakshan et al., 1995) were identified in this way and are expected to be protective in nature.
Drug targets

Current filariasis control strategies are not entirely successful and filarial infections are on the rise. In the absence of availability of antifilarial vaccines, chemotherapy remains the mainstay for treatment of the diseases caused by filarial nematodes.

a) Carbohydrate metabolism enzymes

Carbohydrates play a significant role in providing energy to filarial species. Filarial parasites have active glycogenic and glycolytic pathways and a somewhat submissive tricarboxylic acid cycle (TCA). This is in contrast to mammals, which have active TCA and electron transport systems. Since the catabolic pathways in the parasites differ from those of their hosts, this promises to be an important antifilarial target. The enzymes involved in this pathway like Phospho-fructokinase, Lactate dehydrogenase, Fructose 1,6-bisphosphate, aldolase and other respiratory enzymes responsible for glucose uptake, transport, incorporation and utilization cab be used as antifilarial drug targets (Gupta and Srivastava, 2005).

b) Trehalose metabolism enzymes

Trehalose is a sugar present in many nematode species but absent in mammals. The synthesis, accumulation and utilisation of trehalose by nematodes are important in interaction with their external environment, in osmoregulation, in resistance to desiccation, in cryopreservation and in egg-hatching. Trehalose also functions as a reserve carbohydrate fuel for energy metabolism. The combined action of two enzymes, i.e. trehalose 6-phosphate synthase and trehalose 6-phosphate phosphatase, catalyse the synthesis of trehalose in most organisms. Trehalose metabolism may provide new targets for attacking nematodes parasitic in mammals. In filariids, trehalose has been detected in adult *L. carinii*, *B. pahangi* and *D. viteae* and studies indicate that trehalose is synthesized from glucose (Powell et al., 1986).

c) Chitin metabolism enzymes

Chitinases have been identified in three separate stages in the filarial life cycle and proposed to be a parasite unique target, as the vertebrate host does not contain chitin (Raghavan et al., 1994). In adult females this enzyme plays a role in embryogenesis. However the biological role of this enzyme in Mf and third stage larvae is not yet clear.
d) Lipid metabolism: HMG-CoA reductase

Lipid metabolism is a relatively untouched aspect of the biology of filarial worms. The filarial isoprenoid biosynthetic pathway leads to the formation of quinones, dolichols, geranyl geraniol, juvenile hormones and purine derivatives of isopentenyl pyrophosphate (Comley, 1985). Considering the variety of important biochemical roles attributed to isoprenoids, it is possible that selective inhibition of isoprenoid biosynthesis at the HMG-CoA reductase catalysed rate limiting stage could have drastic consequences on filariae (Gupta and Srivastava, 2005).

e) Protein metabolism: cystathionine-β-synthase

Parasitic helminths are able to take up amino acids from their surroundings through their tegument. However, the identification of a novel, non-mammalian form of cystathionine-β-synthase in nematodes may facilitate the selective inhibition of a parasite-specific enzyme (Walker and Barrett, 1991). Cystathionine synthesizing activity has been found in the filarial nematodes *B. pahangi* and *D. immitis*. Further studies towards non-mammalian serine sulphhydrase activity of cystathionine-β-synthase and its biological role in filarial worms may offer opportunities for selective inhibition (Gupta and Srivastava, 2005).

f) Collagen metabolism: prolyl-4-hydroxylase

The cuticle forms the external surface of parasitic nematodes, thus forming the interface between the parasite and the host. The nematode cuticle consists of a network of collagen molecules, which are primarily held together by disulfide bonds. The central enzyme involved in the biosynthesis of collagen is prolyl-4-hydroxylase which catalyses the post-transcriptional oxidation of proline to 4-hydroxyproline in nascent collagen chains. This enzyme has been a subject of acute concern as a potential chemotherapeutic target, because molecules inhibiting its activity might be expected to be relatively specific inhibitors of collagen biosynthesis (Hanauske-Abel, 1991).

g) Nucleic acid metabolism - DNA topoisomerase II

DNA topoisomerases are the enzymes required for the replication, transcription and recombination of DNA. These enzymes play crucial roles in the organization of DNA within the cell nucleus as well as in its structure and function. Previously some prototype pyrimidine derivative molecules have been
synthesized, which have shown adulticidal and antiinflammatory activities with additional DNA topoisomerase II inhibitory activity. These compounds possessed both macrofilaricidal and microfilaricidal actions combined with sterilizing effect due to DNA topoisomerase II inhibitory activity (Misra-Bhattacharya et al., 2004). In addition some pyrido-indole and quinolone derivatives (Srivastava et al., 2000) and trisubstituted pyrimidine derivatives (Katiyar et al., 2005) were also reported as novel antifilarial agents, exhibiting topo II inhibitory activity.

h) Folate metabolism enzymes

Folate derivatives are concerned with the transport and inter-conversion of carbon units for synthetic reactions. The high synthetic capacities of parasites and the differential sensitivities of certain folate metabolizing enzymes to inhibitors means that folate metabolism is a potential area for chemotherapy. Adult filariae possess an array of enzymes involved in the interconversion of folate analogues (Jaffe and Chrin, 1980) DEC also inhibits a number of folate metabolism enzymes but it remains to be determined whether the ability of DEC to interfere with multiple aspects of filarial folate-related metabolism is in any way related to the antifilarial action of this drug. Suramin inhibits the dihydrofolate reductase of *O. volvulus* and NADP-dependent 10-formyl FH4 dehydrogenase of *B. pahangi* (Gupta and Srivastava, 2005).

i) Biogenic amine metabolism enzymes

Mono- and polyamines are widely distributed in the nature, from prokaryotes to eukaryotes. Parasite-specific putrescine-N-acetyltransferase and polyamine oxidase, both involved in the reversed pathway of polyamine metabolism, were demonstrated for *Ascaris suum* and *Onchocerca volvulus*. Berenil-treatment was found to be correlated with accumulation of polyamines, especially spermine, obviously due to blockaded polyamine interconversion. Furthermore it was shown that added spermine to the culture medium led to the death of worms. These specificities might be exploited for chemotherapy of filarial infections (Müller et al., 1988). The polyamine metabolism of parasites also has attracted increasing interest. The polyamine metabolism of filaria and other helminths is still a neglected area of research, although there are reports about distribution pattern of polyamines and some peculiarities of polyamine metabolism in filarial worms. However DFMO (DL alpha-difluoromethylornithine) and MGBG (methylglyoxal bis-(guanylhydrazone), both of which are potent
inhibitors of polyamine synthesis in mammals, do not significantly effect the viability of filarial worms (Müller et al., 1988).

j) Glutathione metabolism enzymes

Glutathione (GSH) is of major importance in filarial species because it has been proposed to constitute the antioxidant system responsible for the long term existence of filarial worms in mammalian hosts by protecting them from the reactive oxygen species produced by normal metabolism and by immune cells of the host (Brophy and Pritchard, 1994). The GSH as a substrate to various enzymes like glutathione peroxidase (GPX), and glutathione-S-transferase (GST) to quench the free radicals. After GSH has been oxidized to GSSG, the enzyme glutathione reductase (GR) accomplishes the recycling of GSSG back to GSH (Lomaestro and Malone, 1995). Out of all the enzymes that are responsible for the synthesis and breakdown of GSH constitutes the γ-glutamyl cycle. At present only two enzymes of the γ-glutamyl cycle viz. glutamate-cysteine ligase (GCL) and γ-glutamyl trans-peptidase (γ-GT) have been characterized from filarial species (Luersen et al., 2000). Therefore, it seems useful to develop drugs that could selectively deplete or distort glutathione stores in these parasites.

k) Acetylcholine receptors as targets

The most convincing evidence that levamisole acts as a cholinergic agonist at the neuromuscular junction, comes from the study on cholinergic receptors of C. elegans. Further support for a cholinergic mechanism for the paralyzing action of levamisole is provided by the observation that mutants of C. elegans, highly resistant to the paralyzing effects of levamisole, respond very poorly to cholinergic agonists (Lewis et al., 1980). Since excitatory neuromuscular transmission in nematodes is cholinergic, acetylcholine esterase (AchE) is required for the postsynaptic inactivation of acetylcholine. Inhibition of AchE resulted in continued depolarization of postsynaptic junctions with resultant paralysis (Gupta and Srivastava, 2005).

Newer targets from Brugia genome

Mapping B. malayi genes onto the Caenorhabditis elegans protein-protein interaction network reveals an interesting pattern of evolutionarily conserved relations within the context of interconnected functional modules. In addition, defining the molecular mechanisms that allow filarial worms to persist for
decades in an immunologically competent host may yield new strategies for the control of autoimmunity and the management of transplanted tissues. From the Brugia genome sequence data we can identify several systems likely to be fruitful targets for the discovery of additional drug targets (Ghedin et al., 2007).

(i) Molting: The B. malayi genome contains many homologs of genes that encode molecules required for molting in C. elegans (Frand et al., 2005) including proteases, protease inhibitors, nuclear hormone receptors (NRs), cuticular collagens, and chitinases.

(ii) Nuclear receptors: Twenty-seven members of the NR family were identified in the B. malayi genome including orthologs of Ecr (not present in the caenorhabditids) and other NRs acting in the D. melanogaster ecdysone-response cascade.

(iii) Collagens and collagen processing: B. malayi has 82 genes that encode for a collagen repeat (including cuticular collagens and basement membrane collagens), which is less than half the number of collagens found in the C. elegans genome (180). It also encodes enzymes important for cuticular collagen processing such as blisterase-like proteases, protease inhibitors, tyrosinases, mixed-function oxidases, and peptidyl-prolyl isomerase.

(iv) Neuronal signaling: Seven putative biogenic amine heterotrimeric guanosine 5'-triphosphate-binding protein (G protein)-coupled receptors, 44 Cys-loop receptors, and 36 genes encoding potassium channels were identified in B. malayi, a number of which are orthologs of C. elegans genes that can be mutated to give paralytic or uncoordinated phenotypes.

(v) The B. malayi kinome: The B. malayi genome encodes 205 conventional and 10 atypical protein kinases, of which 142 appear to be of fundamental importance based on the severity of their RNAi phenotypes in C. elegans.

(vi) Reliance on host and endosymbiont metabolism: As 9 of 10 enzymes required for de novo purine synthesis, 6 of 7 genes required for heme biosynthesis, and all 5 enzymes required for de novo riboflavin biosynthesis are absent from the B. malayi genome, the worm may be forced to meet requirements for these key metabolic factors by active uptake of host-supplied
molecules or through reliance on wBm, which has complete purine, heme, and riboflavin synthesis pathways (Foster et al., 2005).

However while having a good number of potential targets in the parasite one question that arises is - would the chemotherapeutic blockage of one enzyme alone be adequate to produce the lethal effect?