Chapter 2

2.1. Review and Literature

Lymphatic Filariasis (LF) caused by threadlike worms \textit{W. bancrofti}, \textit{B. malayi} and \textit{B. timori} living in the lymphatic system, belong to Class Chromadorea, Order Spirurida, Super Family Filarioidea and Family Onchocercidae. It is a mosquito borne parasitic disease that is caused by three species of tissue dwelling filaroids i.e. \textit{W. bancrofti} is responsible for 90\% of cases and is found throughout the tropics and sub-tropical areas world-wide (Melrose et al., 2004), \textit{B. malayi} is confined to Southeast and Eastern Asia is responsible for \textasciitilde80\% of the disease, \textit{B. timori} is found only in Timor and its adjacent islands. All three species cause similar disease.

2.1.1. Discovery of Symptoms (1588-1592)

During an exploration of Goa between 1588 and 1592, 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". In 1849, William Prout became the first to document a condition common to LF called chyluria (passage of lymph in the urine so it appears milky) a book entitled ‘On the Nature and Treatment of Stomach and Renal Diseases’.

2.1.2. 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 LF). Three years later, Otto Henry Wucherer discovered microfilariae in urine of a patient 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 these microfilariae and elephantiasis.

2.1.3. 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 now recognize as *W. bancrofti*.

2.1.4. Discovery of the Life Cycle (1877)

Patrick Manson was the first to look for an intermediate host for LF 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.

2.1.5. Discovery of Transmission (1900)

In 1900, George Carmichael Low discovered microfilariae in the proboscis of mosquitoes, and finally pinpointed that transmission is due to an infective bite from a mosquito vector.


2.2. Causative agent

2.2.1. *Wuchereria bancrofti*

Mainly, *W. bancrofti* is responsible for LF infection (about 90%) and remaining 10% caused by *B. Malayi*. Bancroftian filariasis characteristically includes symptoms associated with the genitalia or chyluria in heavily infected patients (John *et al.*, 2006). *W. bancrofti* is transmitted mainly by *Culex* and *Anopheles* and hence microfilaria of *W. bancrofti* is found at high levels at night. Additionally, *W. bancrofti* cannot be maintained in any animal reservoir except humans due to its host specificity. The microfilariae, or larval stage of *W. bancrofti*, are sheathed, and range from approximately 245 to 300 µm. Nuclei do not appear at the end of the tail, which is a major difference from other microfilariae. Infective larvae take several months to become sexually mature. The adult stage can live for several years. “The males range from 2.5 to 4 cm in length, and the females range from 5 to 10 cm in length. One end of the round body is blunt, while the other is pointed. Both bancroftian and brugian filaria lack a digestive system instead absorbing nutrients from their hosts” (John *et al.*, 2006).
2.2.2. *Brugia malayi*

The distribution of *B. malayi* is very similar to that of *W. bancrofti*. However, cases are concentrated in Asia, including Indonesia, Malaysia, South China, Thailand, India, Vietnam, the South Korea and Philippines. Brugian filariasis does not include symptoms associated with the genitalia or chyluria. *B. malayi* is transmitted by *Mansonina* mosquitoes. Since these mosquitoes feed primarily during the day, *B. malayi* microfilaria can be found in the blood during the day. *B. malayi* has been found in Macaques, leaf monkeys, cats and civet cats as well.

The morphology, like that of *W. bancrofti*, is the most reliable way to differentiate species type. Generally, mf range from 200 to 275 \(\mu\)m and adult female worms average about 3.5 to 6 cm long while males are around 1.5 to 2.1 cm in length. Microfilariae of *B. malayi* are sheathed like *W. bancrofti*, and have a very similar shape. However, two
nuclei extend nearly to the tip of the tail, a characteristic not shared with *W. bancrofti* (Strübing et al., 2012; John et al., 2006).

### 2.2.3. *Brugia timori*

*B. timori* is the least common and therefore least studied species of filaria known to cause LF. This species was reported on the island of Timor in 1964, and has been found in other islands in Indonesia. The microfilariae, or larval stage of *W. bancrofti*, are sheathed, and range from approximately 245 to 300 µm. Infective larvae take several months to become sexually mature (Kagai et al., 2013). 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 is only expressed in the lower part of the limbs. However, at approximately 310 µm, *B. timori* microfilaria is slightly larger than that of *B. malayi* (Strübing et al., 2012; John et al., 2006).

### 2.2.4. Bovine Filarial Parasite *S. cervi*

*S. cervi* belongs to the phylum Nemathelminthes and superfamily Filarioidea (Ahmad et al., 2007; Williams et al., 1955). This species occurs in the abdominal cavity of cattle, bison, yak and various species of deer and rarely in sheep. Another closely related species is *S. digitata*, which also inhabits the peritoneal cavity of cattle and other ungulates. *Setaria* species causes cerebrospinal nematodiasis and lumbar paralysis as well as ocular infection in cattle (Ahmad et al., 2007; Shin et al., 2002; Tung et al., 2003). The parasite is generally considered to be non-pathogenic in its natural hosts, but the transmission of the infective larvae through mosquito vectors to its abnormal hosts (goats, sheep or horses) can result in a serious and often fatal cerebrospinal nematodiasis
(Wijesundera et al., 1997). The resemblance of *S. cervi* to various filarial species has been reported earlier (Singhal et al., 1973). The life cycle of filarial parasites encompasses five major stages, delineated by four complete moults of the cuticle (Scott, 2000). Two of these moults occur in the arthropod vector, which takes up blood microfilariae (L₁; mf) and permits rapid development of parasites to the L₃ or infective larval stage. Once L₃s gain access to the mammalian host, they continue developing over many weeks and through two further moults into dioeciously adult worms. After mating, the females release large numbers of mf which continue the cycle when ingested in the blood meal of the arthropod. Each of the major lifecycle stages (mf, L₃ and adults) has unique immunological characteristics. Adult *S. cervi* inhabits the peritoneal cavity of cattle and releases thousands of microfilariae (mf) every day. The adults survive for many years, causing considerable damage to various organs and being responsible for the disease setariasis. They therefore represent a major target in development of novel veterinary medicines (Ansari, 1963). The peritoneal transplantation, periodicity and morphology of mf of *S. cervi* have been studied in white rats (Ansari, 1963, 1964). Suggestions have also been put forward that many neurological syndromes of unknown etiology in humans are due to infection with *Setaria* species (Kadenatsii et al., 1974). Several common antigens between the bovine (*S. cervi*) and human (*B. malayi*) filarial parasites have been demonstrated (Kaushal et al., 1994). *S. cervi* has been widely used as a test organism for the *in vitro* screening of prospective anti-filarial compounds (Ahmad et al 2007; Srivastava et al., 1983a). The *S. digitata, Mastomys coucha* model has also been found to be amenable to chemotherapeutic and immune-biological investigations in experimental filariasis (Srivastava et al., 1983,1984; Srivastava,, 1983b; Mukhopadhyay
et al., 1996). The protein profiles of adult *S. digitata* and *S. marshalli* (Tung et al., 2003) have been compared using SDS-PAGE (Ahmad et al., 2007).

### 2.3. Life Cycle

Filarial nematodes, develop through four larval stages into an adult male or female (Figure), entirely within two host species a mosquito vector (*Culex, Aedes, Mansoni* and *Anopheles*) and humans (or rodent in case of experimental filariasis).

**Figure. 2.2:** The lifecycle of *B. malayi*.

*B. malayi* has a two phase life cycle, passing between the human definitive host and a mosquito vector host (Bogitsh et al., 2012). In the human host, adult males and females reside within the afferent lymphatic vessels just upstream from major lymph node clusters and ovo-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 (Matthews et al., 2011). 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 4 mm 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 3-6 weeks moult twice to become adults, and migrate to the lymphatic system. Microfilarial release occurs after ~100 days, and the adult females can live for 8 years.

Figure: 2.3. Typical filaroid life cycle (CDC/DPDx, used with permission).

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

**2.4.1. Geographical Distribution**

Lymphatic Filariasis is endemic in 81 countries, 66 of these countries have completed mapping their endemic foci 13 countries have made progress in mapping and only 2 countries have yet to start mapping. Mainly in the tropics both north and south (Figure 2.4). India, Indonesia, Nigeria and Bangladesh account for nearly 70% of LF 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). The ‘at-risk’ population for contraction of LF includes 1.2 billion people. Currently, more than 120 million people are affected by LF, including 25 million men who suffer from the genital swellings associated with the disease and 15 million people who suffer from severe lymphoedema or elephantiasis of the leg. (Ningfei *et al.*, 2007)
**Figure 2.4.** LF Endemic countries and territories (Source: The Carter Center)

**Figure 2.5.** Global Distribution of Lymphatic Filariasis
(http://www.the-travel-doctor.com/filariasis.htm)
2.5. Clinical Manifestations

2.5.1. Symptomology and Pathology

After receiving the infective stage larvae (L₃), host immune system show vigorous immune responses to the larvae. If larvae sustain these adversities then various pathologies associated with filarial infection can develop. The nematode parasite alone is not responsible for all adversities but the 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 against 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 then results in chronic manifestations characterized by grotesque deformities and are usually unilateral.

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.
2.5.2. Sequential Development of Filariasis

This may be classified into the following stages:

(i) Biological incubation period (The pre-patent period)

It is the time between entry of infective 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. However, the *B. malayi* takes 3-4 months to develop in the definitive host.

(ii) The patent (symptom-less) period (Secondary or carrier stage)

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 remains
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 (Nutman et al., 2013; Freedman et al., 1999; Dreyer et al., 1992).

(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, 1998). In bancroftian filariasis the ADL attacks occur usually in the limbs and groin while the male genitalia are the most often affected 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 up to 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) The 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 hydrocoele, 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.

### 2.5.3. Categories of 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 $L_3$. However all individuals do not develop into similar state of infection. Following infection with $L_3$, 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. Irrespective of the infection exposure dose and course of development of infection, different individuals respond differently. This is an important feature of human LF that, not all hosts develop microfilaraemia (Lawrence et al., 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.

**Figure: 2.7.** Categories of symptoms present in filarial endemic areas.
(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 (Murty et al., 2013). 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

The individuals with pronounced of these is the damage to the lymphatic vessels, which is mediated by the immune responses to the adult worms living in them belong to this category. These acute immune responses are characterized by lymphangitis (inflammation of the affected area), generalized malaise and fever (Sudhakar et al., 2011). Later the major chronic signs appear as hydrocoele, 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 to 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 (Szuba et al., 2003).
(c) Endemic normal

In most of the endemic areas a portion of the population remains mf negative and is devoid of symptoms of the disease despite lifelong exposure to infection. It is possibly a heterogeneous group consisting of “Truly Immune” cases apart from those with pre-patent or sub clinical or unisexual infections of parasite.

2.6. Disease Management

2.6.1. Diagnosis

Lymphatic Filariasis is 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 (Kagai, 2013). A diethylcarbamazine citrate done, as the treatment with DEC ‘provokes’ the appearance of microfilaria in the blood within 30-45 min of DEC administration, during the day. Hypereosinophilia revealed through the laboratory examination, corresponds to the incidental finding of microfilariae (blood or skin). Infection can be confirmed by the visualization of the embryonic and/or adult parasite. For pathogenic filariasis with microfilaraemia, paradoxically, clinically positive subjects are often amicrofilaraemic. In this case, the presence of antibodies and/or specific serum antigens confirms the diagnosis (Carme, 2007). Filaria dance sign detected in ultrasonography (Amral et al., 2007) lymphoscintigraphy (McCarthy 2000; Palumbo, 2008), based on gamma camera imaging, detects structural changes like lymphatic dilation, dermal back flow and obstruction in oedematous limbs, are the other methods. 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 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) Immuno–chromatic test (ICT) based on antigen detection, applies initially to mapping distribution detects circulating filarial antigen. ELISA-based approach is the alternative for the ICT, using the monoclonal antibody Og4C3. Others include exposure antibodies in children and PCR methods for xenomonitoring filarial parasites in mosquitoes (Palumbo, 2008).

2.6.2. Knott's Concentration Technique

1ml venous blood is drawn from suspected filarial patient and immediately mixed with 10 ml of 2 % formalin solution in a 15 ml centrifuge tube. The sample is thoroughly but gently mixed by tilting the tube up and down a few minutes, then centrifuged at 1500-2000 rpm for 5 minutes. Then supernatant fluid is discarded and smears of the sediment are made of clean glass slides. The smears can be examined wet with cover slip for mf or allowed to dry O/N, stained and observed for mf.

2.6.3. Membrane Filtration Method for Microfilaria Detection

Blood drawn from veins at night and filtered, quantifies load of infection and detection of microfilaria, usually observed before clinical manifestations develop and as lymphoedema develops microfilaria ceases in blood (McCarthy et al., 2000).
2.6.4. Diethylcarbamazine Citrate Provocative Test

Nocturnally periodic microfilariae may be demonstrated in the blood in the day time by the administration of a small dose of diethylcarbamazine citrate (DEC) which moves the mf from the lungs into the peripheral circulation. The blood should be examined 15-20 minutes after an oral dose of 6.0 mg/kg or 45-60 minutes after 2.0 mg/kg of DEC. In LF mf do not appear until at least nine months after exposure to infection. After infection mf rates peaks in the 15-19 year age group in endemic areas but, in the late stages of filarial disease, mf cannot be found in the blood (Manguin et al., 2010). Hence for effective cure

Table 2.1: Diagnosis of parasitological disease

<table>
<thead>
<tr>
<th>No</th>
<th>Test</th>
<th>Merit</th>
<th>Demerit</th>
<th>Acceptability</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Thick Blood smear (20-60 cmm blood) Chandra et al., (1986)</td>
<td>Less expensive</td>
<td>Poor sensitivity</td>
<td>Acceptable for surveys</td>
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<td></td>
<td></td>
<td></td>
<td>Night blood sampling</td>
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<td>2</td>
<td>Counting chamber (20-100cmm blood) Southgate and Desowitz (1971)</td>
<td>Relatively quick</td>
<td>Poor sensitivity</td>
<td>Not fit for field use</td>
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<td></td>
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<td>Night blood sampling difficult</td>
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<td></td>
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<td></td>
<td>Permanent preparation</td>
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<td></td>
<td>Speciation difficult</td>
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<tr>
<td>3</td>
<td>Membrane filtration (1-5 ml blood) Shibuya et al., (1977)</td>
<td>Very sensitive</td>
<td>Expensive</td>
<td>Not fit for field use</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Night blood sampling difficult</td>
<td>Can be used in selected cases</td>
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<td></td>
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<td>Permanent preparation</td>
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<td>Speciation difficult</td>
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<tr>
<td>4</td>
<td>DEC provocation Murthy et al., (1983)</td>
<td>Can be performed in day time</td>
<td>Poor sensitivity</td>
<td>Not fit for mass survey</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>Can be used in selected cases</td>
</tr>
<tr>
<td>5</td>
<td>DNA based diagnostic tests Walther and Muller (2003)</td>
<td>Highly sensitive quick</td>
<td>Cross reaction</td>
<td>Very suitable for field use</td>
</tr>
</tbody>
</table>
and control of filariasis, an early and accurate diagnosis is important (Murthy et al., 1983; Bhumiratana et al., 2004).

2.6.5. Ultrasonography

Recently, the movements of living adult filarial worms of *W. bancrofti* have been observed by ultrasonography in the scrotal lymphatics of asymptomatic males with microfilaraemia. The constant wriggling movement of the adult worms in their ‘nests’ in the scrotal lymphatics has been described as the ‘filaria dance sign’ (Amaral et al., 1994). In contrary, ultrasonography has not helped in locating the adult worms of *B. malayi* in the scrotal lymphatics since they do not involve the genitalia (Shenoy et al., 2000). Moreover, this technique is not proved to be useful in patients with filarial lymphoedema also because living adult worms are generally not present at this stage of the disease.

2.6.6. Lymphoscintigraphy

In lymphoscintigraphy, the structural changes are imaged using a gamma camera after injecting radiolabelled albumin or dextran in the web space of the toes. This method can be directly used to observe lymphatic dilation, dermal back flow and obstruction in the oedematous limbs. Lymphoscintigraphy has shown that even in the early, clinically asymptomatic stage of the disease, lymphatic abnormalities in the affected limbs of people harboring microfilaria may occur (McCarthy et al., 2000).

2.6.7. Polymerase Chain Reaction Based Assay (PCR)

Traditionally, laboratory diagnosis of LF depends on detection of microfilariae in night blood specimens. Although sensitivity has been improved by concentration techniques and using provocative test the traditional parasitological detection methods fail to identify
a microfilaremics or individuals with very low mf levels (McCarthy et al., 2000). Moreover, this time-consuming, labor intensive and tedious method also has difficulty to differentiate one filarial species from another (Poole and Williams, 1990). The efficiency is further decreased by the long pre-patency and nocturnal/ subnocturnal periodicity. Thus, there have been considerable efforts to develop other diagnostic techniques; including immunoassays to detect specific circulating parasite antigens and anti-filarial antibodies, as well as molecular-biology-based assays to detect the parasite DNA. Detection of anti-filarial IgG₄ antibodies enhances the specificity of immunodiagnostic assay for LF (Ottesen et al., 1997; Ottesen, 2008). The advantages of the antibody assays are the cost effectiveness of the tests, high sensitivity and availability for both species of parasite. Recent advances in molecular biology techniques have been employed in designing species-specific primers for PCR-based identification of parasites including diagnosis of LF both in human and animal reservoir blood, as well as in mosquitoes (Table 2.2). For example, PCR techniques based on the identification of repetitive DNA sequences Ssp I (for W. bancrofti) and Hha I (for B. malayi) have been developed. Other PCR based assays, such as DNA hybridization, PCR-restriction fragment length polymorphism (PCR-RFLP) PCR-enzyme linked immunosorbent assay (PCR-ELISA), quantitative PCR and random amplified polymorphic DNA (RAPD) have also proved effective for diagnosis and epidemiological studies of LF.
Table 2.2: Molecular diagnosis for lymphatic filariasis.

<table>
<thead>
<tr>
<th>Filarial sp.</th>
<th>Molecular Technique</th>
<th>DNA target</th>
<th>References</th>
</tr>
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<tbody>
<tr>
<td><strong>B. malayi</strong></td>
<td>DNA hybridization</td>
<td>Hha1 repeat, Hha1 repeat, Glutathion peroxidase gene, ITSI</td>
<td>(Williams et al., 1988)</td>
</tr>
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<td></td>
<td>PCR</td>
<td>Hha1 repeat</td>
<td>(Triteeraprapab et al., 2001a)</td>
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<td>PCR-RFLP</td>
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<td>(Chansiri et al., 2002)</td>
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<td>PCR-ELISA</td>
<td>Hha1 repeat, Glutathion peroxidase gene,</td>
<td>(Nuchprayoon et al., 2005)</td>
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<td>Multiplex –PCR</td>
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<td>(Thanomsub et al., 2000)</td>
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<td>Real time –PCR</td>
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<td>(Mishra et al., 2007)</td>
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<td>(Rao et al., 2006)</td>
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<td>(Thanchomnang et al., 2008)</td>
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<tr>
<td><strong>W. bancrofti</strong></td>
<td>DNA hybridization</td>
<td>pWb 12 repeat, pWb 35 repeat, Ssp1 repeat</td>
<td>(Dissnayak et al., 1991)</td>
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<td>PCR</td>
<td>ITSI</td>
<td>(Sirideva et al., 1994)</td>
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<td>PCR –RFLP</td>
<td>Ssp1 repeat</td>
<td>(Mc Carthy et al., 1996)</td>
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<td>Real time –PCR</td>
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<td>(Nachprayoon et al., 2007)</td>
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2.6.8. Immunodiagnostic tests

The immunological methods such as precipitin and immunodiffusion, complement fixation, immuno and counter-immuno electrophoresis, indirect haemagglutination and flocculation, immunofluorescence, ELISA, chemiluminescent ELISA, radioimmunoprecipitation polyethylene glycol (PEG) assay, immunoradiometric assay (IRMA), intradermal test, cell mediated immunity test, passive cutaneous anaphylaxis, in vitro histamine release assay and immune adherence, etc have been explored to develop
useful test for the diagnosis of different parasitic infections. The different techniques used in parasite serology and their evaluation have been classified based on the reactivity (Voller and de Savigny, 1981; Houba, 1980; Kagan 1981). Gel diffusion, complement fixation and latex agglutination are not useful for detection of antibody within first week of infection because of low reactivity, requiring high concentration of antibody. Tests of 'medium reactivity' are the haemagglutination and immunofluorescence techniques which can detect antibody by the second week of infection. Tests of 'high reactivity' are the radioimmuno assay and ELISA which can detect low concentrations of antibody (nano gram or pico gram/ml) on 3rd or 4th day of infection.

2.6.9. Immunochromatographic Test (ICT)

Card test and ELISA based format are highly sensitive and filarial antigen detection assays, are available for the diagnosis of *W. bancrofti*. But the drawback in the test is their ability only to detect in early stages of disease when parasites are alive (Ho et al., 2013, Weil et al., 1997).

2.6.10. Immuno Diagnosis Based on Antigen Detection

Filarial antigen has been demonstrated in the sera and urine of infected humans and animals using anti-sera raised against heterologous filarial antigens from *L. carinii*, *D. immitis*, *B. malayi*, *S. digitata* (Dasgupta and Shukal Bala, 1978; Desowitz and Una, 1976; Tanabe, 1959; Dissanayake et al., 1984; Hamilton et al., 1984). Few attempts have been made to produce anti-sera against *W. bancrofti* microfilarial antigens. Administration by subcutaneous route was found to be more effective than intravenous injection in eliciting maximum immune response. Kaliraj *et al* have shown that
circulating filarial antigen was concentrated from microfilaraemia plasma by salt precipitation and was identified as an antigen of microfilarial origin using anti rabbit mf sera in CIE (Kaliraj et al., 1979 b). Use of immunoglobulin from chronic filarial serum (FSI) was explored for detection of circulating antigen in filarial sera and culture fluid by CIE and IHAT. FSI was found to be more effective compared to rabbit anti-mf serum in detecting circulating antigen in serum and culture fluid (Kaliraj et al., 1981d; Kharat et al., 1981a). Use of IgG fraction of FSI (FSI-G) in sandwich ELISA was found to be quite sensitive in detecting circulating antigen in 27 out of 33 microfilaraemia sera and an apparent positive correlation between the microfilarial density and the antigen titre was observed (Reddy et al., 1984b). Filarial antigen was also detected in the neat urine samples of microfilaraemia patients by double antibody sandwich ELISA using FSI-G and rabbit anti urinary filarial antigen immunoglobulin and by IRMA using rabbit IgG antibodies to *B. malayi* antigen. Monoclonal antibody is another reagent with great potential for detection of specific antigen of interest. Monoclonal antibodies have been produced to heterologous filarial antigens and *W. bancrofti* mf ES antigens and are being explored for detection of filarial antigen in bancroftian filariasis.

### 2.7. Genomic analysis for filarial parasite

*B. malayi* was selected to be the first parasitic nematode to be fully sequenced despite its relatively minor current importance as a human pathogen because it is the only major filarial parasite of humans that can be maintained in small laboratory animals. Genomic technology is a powerful technology of the bioinformatics which is rapidly being used to understand the structure and function of all the genes within an organism. The field also includes evolutionary studies, intra-genomic phenomena such as heterosis,
epistasis, pleiotropy and other interactions between loci and alleles within the genome (Addiss et al., 2010). Moreover, drug targets can be screened or identified using the genomics approach. Using bioinformatics, approach of phylogenetic analysis (Casiraghi et al., 2001) have identified the gene sequence of mitochondrial cytochrome oxidase-I (COI) from 11 species of filarial nematodes and spirurid nematodes. Recently, nuclear genome draft of the first human filarial parasites of B.malayi (95-Mb) has been reported, which contains 88,363,057bp of assembled sequence with 17.84% of protein coding sequence. The full genome data are available at the recent release of NEMBASE4 database. Analysis of these data using genomics approach can provide significant enrichment in our knowledge to understand the genetic principles that govern various essential functions for their survival which can provide several fruitful drug targets (Scott et al., 2012).

The genome of B. malayi is estimated to be about 100 million base pairs (100 Mb) The repetitive DNA content is 17%, and one single satellite repeat, the Hha I repeat, is present in -30,000 copies (and thus makes up nearly 10% of the genome) (McReynolds, et al., 1986). Other identified repeat DNAs (ribosomal RNAs in the main) make up another 2-3% of the genome. The genome is AT rich (79%) (Rothstein, et al., 1988) and exacerbated in non-coding (intergenic and intronic) regions. The genome is organized as five chromosomes which cannot be separated on pulsed field gels. The Hha I repeat is organized as 8-10 sets of extensive tandem repeats, and it is tempting to suggest that these define regions on each chromosome. These genes were mainly copies of the Hha I repeat and a select set of antigen encoding genes. Many genes had been identified multiple times because of redundancy in (primarily antibody-based) screening methods.
The approximately 22,000 ESTs from *B. malayi* represent about 8800 different genes. Of these, approximately 55% have no significant similarity (a BLASTX score probability greater than e-10) to either *C. elegans* proteins, other known nematode proteins, or to any other sequences from other organisms represented in GenBank.

### 2.8. Therapeutic Approaches to Filarial Infection

#### 2.8.1. Vaccine Development

The different forms of the parasite inhabit different compartments in the mammalian host. Unique set of proteins released by each form reflect particular developmental processes and different strategies for evasion of host responses (Moreno *et al.*, 2008) and thus, provide an important new perspectives on the biology of the filarial parasite in terms of therapeutic and vaccine targets. Individuals living in endemic areas of filariasis require long term chemotherapy in order to prevent re-infection and transmission. However, such strategies are difficult to implement on permanent basis at the population level (Gardon *et al.*, 1997; Molyneux *et al.*, 2005; Tisch *et al.*, 2005). A crucial complementary approach is the development of an effective vaccine against filariasis (Volkmann *et al.*, 2003). Although immunization with recombinant antigens has been actively investigated in filariasis (Fischer *et al.*, 2003a; Wu *et al.*, 2004), the most effective protection is still obtained with irradiated larvae. A variety of animal models have been used to study vaccination against filariasis (Nutman, 2002) and these have relevance to human pathogens because filarial parasites share many biological features including early migration through the lymphatics (Volkmann *et al.*, 2003). Cross-
protection between filarial species suggests that even the antigens identified in animal models will be relevant to human infection (Storey & Al-Mukhtar 1982).

Irradiated $L_3$ vaccinations in the *L. sigmodontis* model to date have involved a challenge with fully infective larvae 2 weeks after their last immunization (Le Goff *et al.*, 1997; Martin *et al.*, 2000). This strategy leads to around 70% reduction in worm burden. The protective effect is established within 2 days and leads to a reduced percentage of microfilaraemic mice 60 days post-challenge inoculation (Le Goff *et al.*, 1997). Vaccine-induced protection is abolished when IL-5 is depleted or genetically absent, as well as in B cell deficient mice (Le Goff *et al.*, 1997; Martin *et al.*, 2000). In addition, degranulated eosinophils are observed at the site of challenge only in mice in which protection has occurred. These data suggest that antibody and eosinophils are the critical players in vaccine-mediated protection.

Intriguingly, the application of anti-helminth vaccines is more advanced in veterinary medicine than in human medicine, with a history of the use of attenuated vaccines against certain nematode parasites and more recently the development of recombinant protein and DNA vaccines against the sheep tapeworm *Taenia ovis* (Drew *et al.*, 2001; Lightowlers *et al.*, 2000). Irradiated $L_3$ larvae have been found to induce protective immunity in rodent models of filarial disease (Bancroft & Devaney 1993; Lucius *et al.*, 1991; Yates & Higashi 1985) and several recombinant $L_3$ antigens have been proposed as potential vaccine candidates (Graham *et al.*, 2001; Gregory *et al.*, 2000). The post-infective stage and $L_3$ to $L_4$ moult are significant developmental stages and the molecules expressed at this time may be of special interest.
2.8.2 Metabolic Enzymes as Chemotherapeutic Targets

Filarial infections are on the rise and current filariasis control measures are not entirely successful. For treatment of the disease, caused by filarial nematodes, chemotherapy remains the mainstay, in the absence of availability of anti-filarial vaccines. However, the precise primary effects of different chemical classes of compounds currently used as anti-filarials are still unclear. “A clear knowledge of the mode of action of anti-filarials awaits greater understanding of the biochemical pathways operating in filarial parasites. 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 comparison to mammals, which have active TCA and electron transport systems. Most adult filarial parasites use the glycolytic breakdown of carbohydrate to lactate as a preferred replenishes to supply their energy requirements. Microfilariae (mf) exhibit an aerobic carbohydrate catabolism, requiring oxygen at least for motility, but apparently not for survival” (Gupta et al., 2005). There are also minor catabolic routes, similar to those present in adults that result in acetate and succinate formation. The complete sequence of glycolytic enzymes involved has been demonstrated with high activity levels in Chandlerella hawkingi, Dirofilaria immitis, Litomosoides carinii, S. cervi, Brugia pahangi, Acanthocheilonema viteae and O. volvulus. Enzymes of the TCA cycle have also been demonstrated in adult B. pahangi, D. immitis, C. hawkingi, Dipetalonema viteae, L. carinii, O. volvulus and S. cervi. However, it was concluded that the TCA cycle does not play a significant role in energy production. The activities of the TCA cycle enzymes were found to be relatively lower in filarial species. Filariae possess mitochondrial electron transport system;
however, the nature of the terminal oxidase is still uncertain. Neither cytochrome c nor was cytochrome oxidase detected in \textit{L. carinii}, \textit{B. pahangi} and \textit{D. viteae}. However, cytochrome oxidase activity has been detected in the \textit{B. pahangi} and cytochromes c, b and a have been detected in adult \textit{D. immitis}. Hayashi and Oya (1978) suggested that \textit{D. immitis} might have a branched cytochrome chain, with an \textit{o}-type cytochrome as the alternative oxidase. \textit{Litomosoides carinii} has a relatively exceptional type of energy metabolism. These filariae show absolute oxygen requirement for maintenance of motility and survival but the TCA cycle does not constitute a significant energy-yielding pathway. Aerobic requirement may reside completely in the oxidative decarboxylation of pyruvate to acetate and CO$_2$.

\subsection*{2.8.3. Glutathione-S-transferase enzyme}

Glutathione-S-transferases are multifunctional proteins that function as enzymes catalyzing the conjugation of glutathione thiolate anion with a multitude of second substrates or non-covalent binding proteins for a range of hydrophobic ligands. Helminths have limited detoxification enzymes and appear to lack the important cytochrome P-450 dependent detoxification reaction. GST has been detected in a range of helminthies, where it may be one of the major detoxification enzymes, and probably plays a role in the survival of the parasite.

\subsection*{2.8.4. Superoxide dismutase enzyme}

Superoxide dismutase (SOD) a prominent scavenger of O$_2$ radical protect the cells from oxidant mediated damage caused by O$_2$ radical. It catalyzes the dismutation to hydrogen peroxide of superoxide radical and molecular oxygen. In recent years SOD and
other antioxidant enzymes (glutathione peroxidase, catalase, glutathione-S-transferase) have been implicated to protect the parasites from the host responses. There is a positive correlation between the levels of anti-oxidant enzymes and parasite survival in the host. Numerous SODs have been characterized, in the parasites of different species e.g, in *Trichinella spiralis*, *Schistoma mansoni*, *Taenia taeniaeformis* and in malarial parasites. *Plasmodium falciparum* and *P. burgei* probably acquired host SOD for its own defence mechanism. SODs of two filarial parasites *Onchocerca volvulus* and *Dirofilaria immitis* have been characterized. Both are Cu-Zn type, an active dimer having molecular weights of 32 kDa and 36 kDa.

2.9. Immune response to filarial infection

Due to long association, immune response of host to parasite in filariasis infection results in 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 (CATO 2005).

2.9.1. Immune response

Elevated level of IgG and IgE were found in bancroftian filariasis compared to their controls (Subrahmanyam *et al.*, 1976). But, specific IgE antibody levels lowered in chronic filariasis, assayed by solid phase radioimmuno assay (Hussain *et al.*, 1981) and enzyme linked immunosorbent assay (ELISA) (Malhotra, *et al.*, 1984b) using *W. bancrofti* and *B. malayi* antigens respectively. But it is interesting to note that antibodies against microfilarial surfaces (sheath) are usually absent in amicrofilaraemia patients when measured using immuno fluorescent assay but these antibodies are found in chronic
filarial patients. When the microfilariae are sonicated then both cuticular and cytoplasmic antigens are produced. Thus antibodies against these antigens were detected in microfilaraemia sera too (Wong and Suter, 1979; Hedge and Ridley, 1977; Kaliraj et al., 1979a). Similarly, the skin test was done using soluble *W. bancrofti* microfilarial antigen and positive reaction (Subrahmanyam et al., 1976) were observed. These anti-microfilarial antibodies was not detected in amicrofilaraemial individuals but were present in chronic filariasis. The microfilarial surfaces were observed to acquire blood group antigens (Ridley and Hedge, 1977) and serum albumin (Maizels et al., 1984) which supposedly help in parasite survival by evading immune system. In chronic filarial pathology cases, usually mf were absent in peripheral blood. Sera from elephantiasis cases promoted an intense adhesion of peripheral blood leucocytes to *W. bancrofti* microfilariae *in vitro*. The adhesion was complement independent and was associated with the IgG fraction in the human system. Antibody-dependent cellular cytotoxicity studies showed the involvement of macrophages and neutrophils as major cell types (Mehta et al., 1981). However, in chronic filariasis, cell mediated immunity was found to be suppressed as observed by lymphocyte transformation assays and leucocyte migration inhibition (Maizels et al., 2003). Th1 cells carry out the protective function against intracellular parasites whereas extracellular parasites are best counteracted by a combination of Th2 and Th1 type cytokines. Some evidence show that naturally immune individuals that are resistance to infection have Th1 cell dominated response (Chensue et al., 1994) Th2 cells are better known for optimal reaction against metazoan parasite with inhibition of Th1 and macrophage development (Pearce et al., 1991; Pearce and MacDonald, 2002; Sher, 1992) in both human and animal models with high level of
tissue eosinophilia, mucosal mastocytosis and elevated IgE level. Th1 and Th2 balanced responses not only provide different modalities of protection against exogenous offending agents but may also play a critical role in the development and or maintenance of the other patho-physiological conditions (Maizels and Yazdanbakhsh, 2003).
Figure No.2.8: Diversity of host's humoral and cellular immune response and the consequences of infection in residents of filarial endemic area.
2.9.2. Heterologous antigens

The non availability of the human parasite (*W. bancrofti*) in required quantity for antigen extraction has become an obstacle for the progress in filaria diagnosis. Hence sharing of antigens (cross reactivity) by different filarial parasites has been exploited in various immunological tests for diagnosis of filariasis. The antigens of *B. malayi* (Grove and Davis, 1978), *S. digitata* and *S. cervi* (Dissanayake and Ismail, 1981; Tandon, et al., 1981), *Litomosoides carinii* (Rao et al., 1980; Dasgupta et al., 1980), *Dirofilaria immitis* (Sawada et al., 1968) and number of other species have been explored for their diagnostic potential for bancroftian filariasis. Gidel et al., 1969 conducted a trial of two immunological tests namely intradermal test and complement fixation test for diagnosis of filariasis using purified antigens from *D. immitis* by Sawada but without success. Studies on the detection of filarial antibody using heterologous antigens were not useful in development of a specific diagnostic test due to significant number of false positive and false negative reactions. Thus, the heterologous antigens have limited or no potential use for immuno diagnosis of filarial infection.

2.9.3. Homologous antigens

Specificity depends on the quality of the antigen employed and acceptable levels of specificity can only be obtained by using homologous and purified antigens. Though, there are some encouraging leads, significant progress is yet to be made in the development of suitable animal model and experimental condition in vitro cultivation of human filarial parasite. Until then infected mosquitoes and humans are the only sources for *W. bancrofti* infective larvae and microfilariae respectively making it difficult to get
required parasite material. Hence studies using homologous (W. bancrofti) antigens are scanty.

2.9.4. Immune complexes

A study of immune complexes is of interest to understand the antigens involved and pathogenic mechanisms in disease processes. Immune complexes were determined by 3% PEG precipitation and complement consumption tests. Significant elevated levels of circulating immune complexes (CICs) were observed in clinical filariasis compared to microfilaraemia and endemic normals. Immunofluorescence assay revealed the presence of mostly IgG and IgM immunoglobulins in the immune complexes (Prasad et al., 1983a; Gajanana et al., 1982). Specific filarial immune complexes have been determined by ELISA using anti C3 and filarial serum immunoglobulin-G (Prasad and Harinath, 1984). The presence of filarial antigen in immune complex was detected by direct ELISA and the involvement of W. bancrofti mf ES antigen was demonstrated by competitive ELISA (Prasad et al., 1983b; Prasad, 1983c).

2.9.5. Host-parasite interactions

Long association of the parasite to the immune system of host in filariasis results in a wide range of clinical and pathological manifestations. To succeed in establishing 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 & Geary 2008). Studies with mouse models confirm that, as in humans, both innate and
adaptive arms of host immunity are targeted by filarial parasites (MacDonald et al., 1998; Taylor et al., 2005).

2.10. Treatment
Several drugs for filariasis have been developed from time to time, diethylcarbamazine (DEC), ivermectin (IVM) and albendazole (ALB) are promising by intensive investigation to be safe and effective filaricidal for mass treatment to control transmission.

2.10.1. Palliative treatment
Pathological symptoms of diseases may be treated at an early stage. There is no drug which can reduce grotesque swelling. Some report is available that DEC with Coumarin can reduce pathological swelling up to some extent. Repeated cleaning of the affected portion with soap and water and application of antibiotic-antifungal creams have dramatic effect on the elephantoid limbs (WHO). Using local antiseptic or antibiotic creams to treat small wounds or abrasions are the Palliative treatment. Asymptomatic should be treated to stop aggravation of the disease symptoms as they have hidden lymphatic damages. Other symptoms like TPE, chyluria are treated with DEC. If there is no cure then surgical treatment is needed.
2.10.2. Diethylcarbamazine (DEC)

Diethylcarbamazine (DEC) is also known as Hetrazan, Banocide, and Notezine. DEC is most promising (Hawking, 1979) and first-line anti-filarial agent for control and treatment of lymphatic filariasis and therapy of tropical pulmonary eosinophilia caused by \textit{W. bancrofti} and \textit{B. malayi} (Ottesen, 1985). DEC drug is effective against both mf and adult worms. This drug markedly lowers the blood mf levels even in single annual doses of 6 mg/kg, and this effect is sustained even after one year. DEC kills the adult worms and their effect is observed only in the 50% of patients. This drug does not act directly on the parasite but its action is mediated through the immune system of the host. The sustained destruction of mf 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 mf 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 \textit{et al.}, 1995). They are usually mild and self-limiting requiring only symptomatic treatment. DEC is the drug of choice in the treatment of tropical pulmonary eosinophilia syndrome in which it should be given for longer periods (3 to 4 weeks). There is also evidence that wide spread use of such drugs, particularly
DEC, is effective in reducing the incidence of clinical lymphoedema (Ottesen, 1985) probably because they can sterilize or kill a proportion of the adult worms.

### 2.10.3. Ivermectin (IVM)

![Structure of Ivermectin](image)

**Figure: 10 Structure of Ivermectin**

Ivermectin (IVM) belongs to the group Avermectin (Macrocyclic lactone) macrolides or macrocyclic lactones produced by actinomycete micro-organisms. Like DEC, IVM drug acts directly on the mf and in single doses of 200 to 400 µg/kg keeps the blood mf counts at very low levels even after one year. The adverse effects noticed in microfilaraemic patient are similar to those produced by DEC but are milder due to the slower clearance of the parasitemia. 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. The drug is now used extensively to control and treat a broad spectrum of infections caused by parasitic nematodes (round-worms) and arthropods (insects, ticks,
and mites) that plague livestock and domestic animals (Campbell, 1993; Campbell and Benz, 1984). IVM is the medicine of alternative against intestinal strongyloidiasis and it is effective against several other human infections caused by intestinal nematodes (de Silva et al., 1997; Gann et al., 1994; Naquira et al., 1989). The agent also has been used successfully against human scabies and head lice. In human beings infected with *Onchocerca volvulus*, IVM causes a rapid, marked decrease in microfilarial counts in the skin and ocular tissues that lasts for 6 to 12 months (Greene et al., 1987; Newland et al., 1988). The drug has little discernible effect on adult parasites, but affects developing larvae and blocks ejection of microfilariae from the uterus of adult female worms (Awadzi et al., 1985; Court et al., 1985). By reducing microfilariae in the skin, it decreases transmission to the *Simulium* black fly vector (Cupp et al., 1986; Cupp et al., 1989). IVM also is effective against microfilaria but not against adult worms of *W. bancrofti, B. malayi, L. loa*, and *M. ozzardi* (de Silva et al., 1997). The drug exhibits excellent efficacy in human beings against *A. lumbricoides, S. stercoralis* and cutaneous larva migrants. Other gastrointestinal nematodes are either partially affected (*T. trichuria* and *E. vermicularis*) or unresponsive (*N. americanus* and *A. duodenale*) (de Silva et al., 1997; Naquira et al., 1989).

### 2.10.4. Albendazole (ALB)

![Structure of Albendazole](image)

**Fig: 2.11 Structure of Albendazole**
Albendazole (Methyl 5-(propylthio)-2-benzimidazolecarbamate) belongs to family benzamidazole. The drug is broad spectrum anti-helminthic excluding Schistosomicides. Appropriate doses are highly effective in single or mixed intestinal helminthic infection.

This anti-helmintic 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., 199).

ALB has no direct action against the mf and does not immediately lower the mf counts. When given in single dose of 400 mg in association with DEC or IVM, the destruction of mf by these drugs becomes more pronounced. ALB combined with DEC or ivermectin is recommended in the global filariasis elimination programme.

2.10.5. 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. This not only prevents transmission of filariasis in the community by reducing the mf levels, but also has the added benefit of clearing the intestinal helminthes (Shenoy et al., 200). ALB added to single-drug therapy with IVM or DEC against LF was in-conclusive results. None of the drug combinations against LF showed more adverse reactions than single-drug therapy (Olsen et al., 2007).