2. Review of literature
2. REVIEW OF LITERATURE

2.1 General introduction of the disease

Members of the Phylum Arthropoda are widely distributed of animal groups. Particularly, those within the classes Insecta and Arachnida live in close association with humans. The class Insecta form the largest of all animal classes with about 1 million described species. The most important form of arthropods is the blood-sucking habits of ectoparasites such as mosquitoes, bedbugs and fleas. Mosquitoes comprise of about 3200 species occur worldwide. They act as vectors of various parasites, which are pathogenic to human. More significantly blood-sucking insects transmit the organisms, hence are pathogenic/parasitic called as “vectors” causing several debilitating diseases such as filariasis. and sometimes fatal diseases such as malaria, dengue, Japanese encephalitis, plague, typhus, yellow fever etc.

Filariasis is a chronic, debilitating and often disfiguring disease widely distributed in the tropical and subtropical regions of Africa, Asia and Northern South America, Western Pacific and Eastern Mediterranean. It is a group of human and animal infectious diseases caused by nematode parasites generally called “Filariae” that include several hundred species of worms that are slender and elongated and
Parasitic in tissues of various vertebrate hosts. There are also about 500 species in this family occurring both in humans, and in domestic animals, rodents, monkeys, lizards etc. Parasites known to cause human infections belong mainly to the genera, *Wuchereria, Brugia, Onchocerca, Dipetalonema, Mansonella, and Loa*. They reside either in lymphatics or muscles, connective tissues, body cavities etc., of vertebrate hosts. They may be classified into the three main groups based on the habitat of the adult worm i.e., is the cutaneous group, the lymphatic group and the body cavity group. Based on the habitat of the adult worms few of the filarial species infecting man and the disease caused by them with their intermediate hosts are listed in the Table 1. The infection is transmitted by intermediate hosts which are always blood-sucking arthropods of the order Diptera, belonging to the four families and some of the known vectors of human filariae are given in Table 2. Only two genera, *Wuchereia* and *Brugia*, are mainly responsible for human lymphatic filariasis. There are two species of *Wuchereria* and nine of *Brugia* (Mak, 1983) listed in the Table 3. Common animal parasites are *Setaria digitata*, and *S. cervi*, (bovine), *Dirofilaria immitis* (dog), *D. uniformis* (rabbit), *Litomosoides carni* and *Dipetalonema viteae* (gerbil), *Brugia pahangi* (cat), *Acanthocheilonema viteae* (jird).
Table: 1 Human filarial species and the habitat of adult worms

<table>
<thead>
<tr>
<th>Species</th>
<th>Habitat</th>
<th>Intermediate host</th>
<th>Disease caused</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>W. bancrofti</em></td>
<td>Lymphatics</td>
<td>Mosquito sp</td>
<td>Bancroftian filariasis (Elephantiasis)</td>
</tr>
<tr>
<td><em>B. malayi</em></td>
<td>Lymphatics</td>
<td>Mosquito sp</td>
<td>Malayan filariasis</td>
</tr>
<tr>
<td><em>B. timori</em></td>
<td>Lymphatics</td>
<td>Mosquito sp</td>
<td>Timor fever</td>
</tr>
<tr>
<td><em>Loa loa</em></td>
<td>Connective tissue</td>
<td>Chrysopsis sp</td>
<td>Loasis</td>
</tr>
<tr>
<td><em>Mansonella ozzardi</em></td>
<td>Serous membranes</td>
<td>Culicoides sp</td>
<td>Ozzard’s filaria</td>
</tr>
<tr>
<td><em>Onchocerca volvulus</em></td>
<td>Skin</td>
<td>Simulium sp</td>
<td>Onchocerciasis (River blindness)</td>
</tr>
</tbody>
</table>

Table: 2 Vectors of human filariae

<table>
<thead>
<tr>
<th>Order</th>
<th>Family</th>
<th>Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diptera</td>
<td>Culicidae - Mosquitoes</td>
<td><em>Cx. quinquefasciatus</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Mansonia sp</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>A. barbirostris</em></td>
</tr>
<tr>
<td></td>
<td>Simulidae - Black flies</td>
<td><em>Simulium damnosum</em></td>
</tr>
<tr>
<td></td>
<td>Certopogonidae - Biting midges</td>
<td><em>Culicoides furens</em></td>
</tr>
<tr>
<td></td>
<td>Tabanidae - Horse flies</td>
<td><em>Chrysopsis dimidiata</em></td>
</tr>
</tbody>
</table>
Table: 3 Nematode parasites causing lymphatic filariasis: Recorded species of *Brugia* and *Wuchereria* parasites

<table>
<thead>
<tr>
<th>Genus <em>Brugia</em> Buckley, 1960</th>
<th>Genus <em>Wuchereria</em> ilva Aranjo, 1877</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>B. malayi</em> (Brug, 1927)</td>
<td><em>W. bancrofti</em> (Cobbold, 1877; Seurat, 1921)</td>
</tr>
<tr>
<td><em>B. pahangi</em> (Buckley and Edeson, 1956)</td>
<td></td>
</tr>
<tr>
<td><em>B. patei</em> (Buckley, Nelson and Heisch, 1958)</td>
<td><em>W. kalimantani</em> (Palmieri et al., 1980)</td>
</tr>
<tr>
<td><em>B. (Brugiella) buckleyi</em> (Dissanaike and Paramanathan, 1961)</td>
<td></td>
</tr>
<tr>
<td><em>B. ceylonensis</em> (Jayewardene, 1962)</td>
<td></td>
</tr>
<tr>
<td><em>B. guyanensis</em> (Orihel, 1964)</td>
<td></td>
</tr>
<tr>
<td><em>B. beaveri</em> (Ash and Little, 1964)</td>
<td></td>
</tr>
<tr>
<td><em>B. tupaiæ</em> (Orihel, 1966)</td>
<td></td>
</tr>
<tr>
<td><em>B. timori</em> (Partono et al., 1977)</td>
<td></td>
</tr>
</tbody>
</table>
LF is primarily a disease of poor because of its prevalence in disfavoured peri-urban and urban areas and also in rural areas. Of all, LF is the second leading cause of permanent and long-term disability. Of the 120 million infected globally in various countries (Fig. 1) an estimated 118 million people have one form or other of the clinical disease and 74 million are microfilaraemic but asymptomatic, another 27 million men are believed to have hydrocoele due to filariasis. In addition, approximately 16 million have lymphoedema or elephantiasis along with the recurrent episodes of acute adenolymphangitis (ADL). Lastly, a million individuals have cryptic infections resulting in conditions such as tropical pulmonary eosinophilia (TPE) (WHO, 2002). In India, *W. bancrofti* and *B. malayi*, are the only two filarial species causing lymphatic filariasis and the former being the major contributor and the latter is endemic to a few parts of Kerala, Tamil Nadu, Andhra Pradesh, Orissa, West Bengal, Assam and Madhya Pradesh (Raina *et al.*, 1995). Figure 2 shows the distribution of filariasis in India based on the filarial endemicity rates ranging from non-endemic to highly endemic areas (Sabesan, 2000).

### 2.2 History of filariasis

It is one of the oldest and most debilitating diseases in the world. The history of this disease is as old as the history of human
Fig. 2 Filariasis Distribution in India - 1995
Based on Historical Data (1960 - 1995)

Source: Sabesan et al., 2000
civilization. Indications on the occurrence of elephantiasis can be obtained from the statue of king "Menthu-hoptep" in Egypt as early as 2133-1992 B.C. People with elephantiasis were excluded from the Buddhist priesthood during 600-250 B.C. (Laurence, 1967). Historical records of this disease seen in 7th – 8th century A.D. is given as an illustration in ‘Yamai’zoshi’ of elephantiasis leg in a woman (Fig. 3) which is preserved in the Tokyo National museum (Tada et al., 1999). Also in Cairo museum the statue of an Egyptian Pharaoh that depicts the signs of elephantiasis (Fig. 4) is seen even today (Dean, 2001). The mummified body of Natsef-Amun, a priest at Karnak in the time of Rameses XI during 1113-1085 B.C. was proven by autopsy to have lymphatic filarial worms in the groin even after 3,000 years (Fig. 5) and is still preserved in Leeds museum (Dean 2001). The Operational Manual published in 1995 by National Malaria Eradication Programme on National Filaria Control Programme, India reports that in as early as 600 B.C. Susruta, a physician mentioned about the disease as ‘Sleepad’. Later in 70 A.D. Madavakara, a pathologist in his book “Madhava Nidan” described the signs and symptoms of this disease (Sharma, 1995). And these signs and symptoms of this disease stand true even today. NMEP, 1995 further reports that Clark (1703) seemed to have described the elephantoid legs in Cochin as ‘Malabar Legs’. During 16th century elephantiasis of the leg attributed to the
Fig. 3 An illustration in 'Yamai'zoshi' of leg elephantiasis in a woman approximately 700-800 years ago, which is preserved in the Tokyo National Museum.
Fig. 4: Cairo Museum: Statue of an Egyptian Pharaoh depicting possible signs of elephantiasis. Source: GSK

Fig. 5: The mummified body of Natsef-Amun, a priest at Karnak in the time of Rameses XI (1113-1085 B.C.) proven after 3,000 years by autopsy to have LF worms in the groin. Source: Leeds Museum
curse of Saint Thomas, Kerala (South-West India) is being portrayed by Linschoten, which is certainly the *B. malayi* infection (Fig. 6) (Laurence, 1989). The dramatic symptoms caused by the infection of *W. bancrofti*, especially the enormous swelling of legs or scrotum were recorded in much of the old and ancient medical literature of India, Persia, China, Japan etc. During the 18th century a Jesuit missionary in Pondicherry, Southeast India portrayed the elephantiasis of the scrotum (Fig. 7) and published in Paris, possibly *W. bancrofti* infection that indicates the possible existence of the disease in Pondicherry, Union Territory - South India (Laurence 1989).

2.3 Life cycle of lymphatic filarial parasites

Lymphatic filarial parasite requires two hosts the definitive host either man or vertebrate animal (e.g. monkey, cat, rat, and jirds) and the intermediate host being mosquito to complete its life cycle (Fig. 8). The life cycle of *Wuchereria* and *Brugia* are essentially similar. Adult worms normally live in the lymph canals and lymph nodes. The female filarial worms in the mammalian host mate with males and produce embryos called the mf which appear in the circulating blood, or occasionally in hydrocele fluid or chylus urine. The mf when ingested by mosquito intermediate hosts, lose their sheaths in the mosquito midgut and migrate to the thoracic muscles to develop to the first stage
Elephantiasis of the leg attributed to the curse of Saint Thomas, Kerala, SW India 16th century, as portrayed by Linschoten. Almost certainly *B. malayi* infection.

Fig. 6

Elephantiasis of the scrotum, portrayed by a Jesuit missionary in Pondicherry, SE India, 18th century, and published in Paris. Possibly *W. bancrofti* infection.

Fig. 7
Fig. 8 Life cycle of lymphatic filarial parasite and its vector
larvae (L1), then to the second stage larvae (L2) and finally to the infective stage (L3) in about 2 weeks. The L3 then migrate to the head of the mosquito and get transmitted to the mammalian hosts during the subsequent feeding (WHO, 1984). During mosquito feeding, L3 are deposited on the skin surface near the site of the puncture wound and actively migrate into the puncture wound and then into the lymphatic system (Mak, 1983). The infective stage larvae develop into adult worms within the human body and it is assumed that about one year period is required for the larvae to grow into adult worms, mate, and produce mf in human hosts which then circulate in blood (Plate 1) to be picked up by the vector again (Sasa, 1976).

2.4 Periodicity of microfilariae

The mf of both the species of human filariae *W. bancrofti*, *W. kalimantani* circulate in the blood in large numbers at night and almost disappear during the day being confined to the lung region. This phenomenon is called nocturnal periodicity of microfilariae. However those of sub-periodic strain appear during the day time exhibiting the maximum number of mf for a specific time of the day.
Plate 1: Microfilaria of *Wuchereria bancrofti* as seen in blood smear stained with Giemsa stain $\times 1000$
2.5 Clinical manifestations of LF

Lymphatic filariasis is not a fatal disease, but is grossly disabling and disfiguring disease (Dean, 2001). Though the infection mainly and often occurs in childhood, the symptoms are commonly delayed until adulthood (WHO, 2001). The disease exhibits broad clinical spectrum (Fig. 9) ranging from asymptomatic to chronic lymphatic dysfunction resulting in elephantiasis and/or hydrocele.

Fig. 9 Clinical spectrum of lymphatic filariasis.

Depending upon the endemicity of the area the proportion of unexposed, exposed and infected people varies as shown in the following figure 10.
Parasite

Host

Responsive (appropriate)

Hypo-responsive (tolerant/suppressed)

Responsive (inappropriate)

Hyper-Responsive

i. EN - Immunity

ii. MF positive

Pathology:

iii. ADL

iv. Elephantiasis

v. Unusual Pathology TPE

**Fig. 10** Host’s immune response and the consequences of infection.

i) Where there are individuals with no signs of infection, or disease generally called as Endemic normals (EN),

ii) Asymptomatic microfilaraemic persons with microfilaraemia known as mf carriers,

iii) Group of individuals who develop signs of responsiveness against adult worms with fever and adenolymphangitis (ADL),

iv) Develop chronic pathology latter (chronic patients)
v) And in individuals with symptoms of tropical pulmonary eosinophilia (TPE) there is a vigorous immune response directed against the microfilaria with consequent pathology.

Clinical manifestations of lymphatic filariasis associated with adult or developing adult worms usually begin in late childhood or early adulthood. Manifestation of the disease is characterized by acute ADL usually accompanied by fever, and chronic obstructive lesions that develop years later with repeated acute attacks. The variety of clinical manifestations include lymphoedema [swollen appearance to the limb which leads to elephantiasis of the limbs (swelling of the limb like the elephants leg (Plate 2)], hydrocoele [enlargement of the scrotum (Plate 3)], chyluria (appearance of lymph fluid in the urine, with or without blood) as well as genetal diseases such as the enlargement of the genitals, vulva (Plate 4) and enlargement of the entire leg or arm, the breasts (Plate 5) or penis. Lymphoedema is generally the first sign of chronic lymphatic filariasis. This condition develops gradually, as the remnant of an attack of acute lymphangitis. According to classification made by International Society of lymphology in 1985 filarial lymphoedema is of three types.
Plate 2: Elephantoid leg of a patient in chronic manifestation of bancroftian filariasis.

Plate 3: A case showing hydrocoele and lymphoedema in bancroftian filariasis.
Plate 4: Enlargement of vulval region in bancroftian filariasis

Plate 5: Enlargement of breast in bancroftian filarial infection.
**Grade I Lymphoedema**

Mostly pitting type of oedema with some fibrosis, which is spontaneously reversible on elevation.

**Grade II Lymphoedema**

Mostly non pitting oedema with fibrosis and not spontaneously reversible. The adjective mild, moderate and severe can be applied to this category.

**Grade III Lymphoedema (Elephantiasis)**

A monstrous increase in volume as described in grade II Lymphoedema with dermatosclerosis and papillomatous outgrowth.

The other symptoms include TPE (also referred to as occult filariasis), filarial fever, epididymo-orchitis (swelling and pain in the testes), funiculitis (painful swelling of the spermatic cord), lymphadenitis (swollen lymph nodes), abscess, lymphangitis, lymph varix, lymph adenovarix, lymph scrotum, lymphorrhea and lymphuria. Infection can also lead to acute, recurrent secondary bacterial infections known as acute attacks.

Filariasis due to *W. bancrofti* and *B. malayi* differs from other types of filariasis where the adult worms reside in the lymphatic systems and cause various symptoms resulting from acute or chronic
inflammation and the blockage of lymph canals. In *W. bancrofti* infection these appear in the form of swelling of the legs or hands or both resulting in elephantiasis, hydrocele and chyluria. In *B. malayi* infection also swelling of legs or hands or both (Plate 6) occurs and the urinary and genital systems are rarely affected though elephantiasis is common. In case of bancroftian filariasis, elephantiasis will be extended beyond the knees to affect whole of each leg whereas in *B. malayi* infection, swelling is restricted below the knees. Mortality due to filariasis is negligible, but there is a high degree of morbidity due to its acute and chronic manifestations.

The socio, economic aspect of the disease has been given importance in the last few years and it was reported that the person having chronic filariasis suffers from a social stigma and the affected person tend to be segregated from the society (Sharma, 1995). People with hydrocele are the subject of considerable teasing (Lu et al., 1998). The disease reduces the people’s ability to work resulting in loss of family income. The global burden of LF was estimated to cause a loss of about a million Disability Life Adjusted Life Years (DALYs) (Datta, 2000). In India alone the economic losses resulting from decreased productivity and lost workdays are estimated to be of the order of US$ 1 billion annually (WHO, 2002).
Plate 6: Clinical manifestation of lymphatic filariasis showing swelling of both the legs and one of the hands in Malayan filariasis caused by *B. malayi*
2.6 Immunological aspects of lymphatic filariasis

LF is a mosquito-transmitted nematode infection caused by nematode parasites. The pathogen has complex life cycle involving a number of discrete stages, which tend to occupy different parts of the body and more importantly they are antigenically different. Till now a clear understanding of parasite physiology, host-parasite relationship, mode of action of drugs, mechanism of drug resistance, patho-physiology and immune response of the disease is lacking. Also, till date the role of immune response in either protection against infection or inducing the pathology is not clear.

The disease exhibits diverse humoral and cellular immunological response in individuals residing in filarial endemic areas. Figure 11 illustrates the spectrum of immunological response of the individuals residing in an endemic area. Although all exposed host individuals mount detectable immune responses to the parasite, the nature and intensity of these responses vary in relation to the host's pathological and clinical status. Those individuals subjected to continued parasite transmission but remain infection free form a particularly important endemic normals group called ENs and can be assumed as putatively immune individuals. These people have the ability to kill/inhibit growth and development of infective larvae, adult worms and/or mf. Up to now it remains unclear as how this type of immunity against infection is
Fig. 11 Diversity of host’s humoral and cellular immune response and the consequences of infection in residents of filarial endemic area. (Ottesen, E. A 1980, King & Nutman, 1991; Ravichandran et al., 1997.)
generated and what are the components of host immune system that participate in the induction of this immunity in this group of people exists.

Another group of individuals who are asymptomatic microfilaraemic persons termed to be tolerant of the parasite and exhibit a down regulation in the immune response. Yet another group of individuals who develop signs of responsiveness against adult worms with fever and develop chronic pathology later. Finally there are several unusual conditions of clinical hyper-responsiveness associated with filarial infections and one such syndrome is TPE (King & Nutman, 1991).

Humoral immune response in filariasis has been assessed through quantitative measurement of specific antibody in sera. Wamae et al. (1995) reported that endemic normals were found to have high IgG1 type of antibody and the study by Thomas et al. (1993) reports that mf carriers had high levels of IgG4 antibodies. Chronic patients were found to have very high level of IgE. The specific antibody titres of IgG, IgM, IgE were typically reduced in mf carriers compared to endemic normals and chronic patients (Piessens et al., 1987: Ottesen et al., 1982). With regard to cellular immune response the predominance in Th1 type cytokine (IFN γ, IL-2) production in EN individuals leads
to resistance to infection and the predominance of Th2 type cytokine (IL-5, IL-10, IL-4) production leads to progressive and persistent infection (Ravichandran et al., 1997). But till now the mechanism by which the shift in response towards Th2 predominance in mf carriers is unclear and any antigen which induces the Th1 type response or which reverts the Th2 response to Th1 could be used for immunoprophylactic studies (Ravichandran et al., 1997).

2.7 Diagnosis of the disease

Diagnosis of filariasis is normally based on parasitological and clinical features and recently based on immunological and molecular methods. Certain clinical features such as swelling of the lower limbs, scrotal region and episodic fever etc., are also considered for the diagnosis of the disease. Parasitological diagnosis is based on the detection of mf stage of the parasite in blood collected at night and the immunological diagnosis includes the detection of antibody or antigen in blood, plasma, sera and other body fluids such as urine and hydrocele fluid. Molecular diagnosis is based on the detection of parasite DNA in human blood or in the vector. Following are the various methods and their detailed procedures and their merits and demerits to diagnose the disease.
2.7.1 Parasitological methods for the detection of the parasites

**Direct examination** (*Manson-Bahr & Bell, 1987; Chatterjee & Singh, 1994*)

Detection of microfilariae in thick blood smears is the common method employed for diagnosis of the infection. This is carried out by collecting blood smears from individuals during 21.00-23.00 hrs by finger prick method and examining them for the presence of mf after Giemsa's staining (Plate 1). However, this method has several drawbacks such as: i) difficulty to detect low level of microfilaraemia and ii) requirement of night blood due to nocturnal periodicity of microfilariae causes great inconvenience.

**Knott's Concentration Technique** (*Mak, 1983*)

One ml of venous blood is taken from the patient and immediately mixed in 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 times, then centrifuged at 1500-2000 rpm for 5 minutes. The supernatant fluid is discarded and smears of the sediment are made on 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.
Nucleopore Membrane Technique *(Dennis & Kean, 1971)*

One ml of heparinised blood is diluted with 9 ml of Tris-Ethylene Diamine Tetra Acetic acid (EDTA) Borate Saline (BS) [TEBS] in a 10 ml syringe. This is slowly passed through the nucleopore polycarbonate membrane (5μm pore size) filters assembled in the holders. A further 10 ml of TEBS is slowly passed through the membrane. Then the holder assembly is carefully detached and the membrane removed. The membrane is placed onto a glass slide and then dried. The dried membrane is fixed for 30 seconds with methanol, washed by dipping momentarily in water and stained with Field’s stain. The stained membrane is examined directly under a microscope or mounted in a drop of immersion oil with coverslip before examination.

However, the above two methods are not considered for routine diagnosis as these techniques need venous blood which has poor community acceptance.

Diethylcarbamazine citrate provocative test *(Manson-Bahr & Bell, 1987; Chatterjee & Singh, 1994)*

Nocturnally periodic mf may be demonstrated in the blood in the day time by the administration of a small dose of diethylcarbamazine citrate which flushes the microfilariae from the lungs into the peripheral circulation. The blood should be examined 15 minutes after an oral dose of 6.0 mg/kg or 45-60 minutes after 2.0 mg/kg.
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 (Manson-Bahr & Bell, 1987). Hence for effective cure and control of filariasis, an early and accurate diagnosis is important.

2.7.2 Immuno-diagnostic techniques

The two main approaches of immunodiagnosis are antibody and antigen detection assays in test samples. In the development of immunodiagnostics for filariasis the two major problems being faced are lack of parasite material especially in bancroftian filariasis because of the absence of successive animal model and the lack of understanding of the dynamics of the disease in terms of antibody production. This problem can be solved by using antigens from closely related parasites such as the homologous filarial parasite *B. malayi* sub-periodic form which can be maintained easily in rodent models (Ash & Riley, 1970) and heterologous filarial parasite such as *Setaria cervi* (Kaushal & Kaushal, 1995) as a source of antigen. Various types of antigens available for this purpose include either of circulating mf antigens, excretory/secretory (e/s) antigens, recombinant antigens or polyclonal and monoclonal antibodies (MAbs) raised against them.
2.7.2.1 Antibody detection in filariasis

Antibody detection helps to study the exposure to infection and the immune response in human and several workers have attempted to develop filarial specific antibody assays.

Antibody detection assays

Various tests have been described using homologous and heterologous filarial parasites as a source of antigen. These assays were performed mainly by using surface, somatic, e/s and recombinant antigens. Gel-diffusion (GD), counter-current-immunoelectrophoresis (CCIE), indirect fluorescent antibody test (IFAT) and enzyme linked immunosorbent assay (ELISA) and Hemagglutination inhibition assay (IHA) (Kagan, 1983; Harinath 1984; Kaliraj et al., 1981; Kharat et al., 1982) are some of them. Many workers in the past have used B. malayi antigens for immunodiagnosis of bancroftian filariasis, especially for antibody detection. Among them are the IFAT, which is routinely being used for the serological diagnosis of filariasis using papain-treated mf, sonicated mf and adult worm sections of sub-periodic B. malayi (Mak, 1983). Parab et al. (1990) used B. malayi microfilarial extract for the detection of antibodies and antigens from endemic residents of bancroftian filariasis. Harinath et al. (1996) used B. malayi mf e/s antigens in the detection of filarial IgG antibodies and
reported that filarial IgG antibody positivity was 72%, 68% and 54% respectively in generalised, cervical and inguinal lymphadenopathy cases and only 28% in epididymorchitis cases. In dot-ELISA using *B. malayi* adult antigen Tandon *et al.* (1988) were able to detect the disease in most symptomatic and asymptomatic patients of filarasis based on antibody detection. Cheirmaraj *et al.* (1991) used *B. malayi* adult soluble fraction-6 to detect filarial antibodies in microfilaraemic (n=522) sera. Among *B. malayi* antigens, adult worm antigen appeared to be more discriminatory and more specific than other stages of antigens for the detection of filarial antibody (Kaushal *et al.*, 1984).

Few other researchers have used e/s antigen of mf of *W. bancrofti* to detect antibodies in the diagnosis of filariasis (Kharat *et al.*, 1982; Malhotra & Harinath, 1984) Simplified ELISA using cellulose acetate membranes in the form of disk/stick was found convenient for field use (Parkhe *et al.*, 1986). However, antibody detection was proved to be less specific as it could not differentiate between active infection and past infections in a filarial endemic area (Reddy *et al.*, 1984; Rajasekariah *et al.*, 1991; Padigel *et al.*, 1995). Among various assays developed ELISA is most effective and sensitive enough in detecting filarial antibodies.
2.7.2.2 Antigen detection in filariasis

The main drawback in antibody detection is that they do not distinguish between current and past infection intensity i.e., the number of worms in the patient. Only the antigen detection can indicate the active infection and differentiates from chronic or past infection. In view of this, steps have been taken to develop a suitable diagnostic method for detection of filariasis which should determine microfilarial antigen(s) for the diagnosis of microfilaraemia.

Polyclonal antibody based antigen detection assays

Several laboratories have developed polyclonal antibody based assays to detect filarial antigens in serum, urine, hydrocele and other body fluids of individuals residing in endemic areas. They are:

Detection of filarial antigen in serum

Several antigen assays were developed using filarial specific immunoglobulin in formats such as Counter Immuno Electrophoresis (CIEP) (Weil et al., 1984: Weil & Liftis, 1987) sandwich ELISA (Reddy et al., 1984, 1986: Santanam et al., 1989), Immuno Radiometric Assay (IRMA) (Hamilton et al, 1984, Paranjape et al., 1986: Forsyth et al., 1985), Radial Immuno Poly Ethylene Glycol Assay (RIPEGA) (Hamilton et al., 1984a) and immunoblot (Weil et al., 1990), stick inhibition ELISA (Ali khan et al., 1990) are some of
the examples. Cheirmaraj et al. (1992) raised polyclonal antibodies in mouse ascitic fluid against *B. malayi* adult SDS soluble antigen, mf SDS soluble antigen (1992) and used them to detect filarial antigens in individuals. When fractions Bm A-6 and the anti Bm A SDS-soluble antigen antibodies developed by Cheirmaraj et al. were evaluated for their diagnostic use in detecting filarial antigen in serum by inhibition ELISA, filarial antigen could be detected in about 85% of mf, 35% clinical filariasis and up to 26% of endemic normal sera samples. (Chenthamarakshan et al., 1995). *B. malayi* mf e/s antigen BMEDEI was used by Chenthamarakshan et al. (1996) through inhibition ELISA with its antibodies and it showed 88% sensitivity and 89% specificity in detecting the antigen. Also for FsIg and *B. malayi* mf e/s antigen, penicillinase was used in stick penicillinase ELISA by Harinath et al. (1996) and reported that filarial antigen positivity ranged between 40 to 80% in acute, chronic and occult filarial cases. Several other antigen assays developed include the ELISA system developed by Lunde et al. (1988) to detect *W. bancrofti* antigen in the circulating immune complexes. The test detected 10 of 28 patients with bancroftian filariasis residing either the Cook Islands (sub-periodic form of *W. bancrofti*) or in India (periodic form of *W. bancrofti*). This test system showed only 42.8% specificity that may be due to the strain variation in different geographical variation.
Cheirmaraj et al., 1992 raised polyclonal antibodies in mouse ascitic fluid against *W. bancrofti* microfilarial antigens and tested for their usefulness in detecting the *W. bancrofti* antigen in sera by sandwich and dipstick ELISA which showed 93% sensitivity.

Also for FSG and *B. malayi* mf e/s antigen, penicillinase was used in stick penicillinase ELISA by Harinath et al. (1996) and reported that filarial antigen positivity ranged between 40 to 80% in acute, chronic and occult filarial cases.

**Detection of filarial antigen in urine, hydrocele**

Detecting filarial antigen in urine and in hydrocoele fluid collected from infected patients also was carried out by several workers. Ramprasad & Harinath (1985), Malhotra et al. (1985) developed an assay for detection of antigens in urine and for detecting in hydrocele fluid and reported that the assays provided accurate means of detecting infection than detection of antibodies. Using antibodies raised against *B. malayi* adult antigens Reddy et al. (1984) detected the presence of filarial antigen in 6 out of 10 urine samples collected from microfilaraemic patients and in 1 out 5 urine samples obtained from clinical cases who resided in *W. bancrofti* endemic areaes. This assay had the sensitivity of 60%. Malhotra et al., (1985) detected *W. bancrofti* filarial antigen using concentrated urine as antigen collected from the
patients in urine samples obtained from 3 of 21 endemic normals, 36 of 42 patients with mf, 17 of 21 with clinical filariasis and in 19 out of 25 hydrocoele fluid samples by sandwich ELISA. In this assay the sensitivity in detecting urinary filarial antigen was found to be 85% and in clinical filariasis and in hydrocoele fluid the sensitivity was found to be 80% and 76% respectively and the specificity of the assay was found to be 85.7%. Padigel et al. (1995) developed a sandwich ELISA by using anti-UFAC2-DE1 antibodies filarial antigen and the assay detected filarial antigen in urine of about 90% microfilaraemics 30% clinical and 10% of endemic normals residing in Sevagram, Maharashtra and surrounding villages which are endemic for nocturnally periodic form of *W. bancrofti*.

Hence, in the present study, the possibility of using polyclonal antibody raised against *B. malayi* to detect *W. bancrofti* antigen present in sera samples of the patients residing in bancroftian filarial endemic area is explored. The assay was developed using *B. malayi* antigens for immunodiagnosis of bancroftian filariasis. In our study we examined the ability of rabbit and mouse immunoglobins against the soluble antigens of mf of *B. malayi* in identifying the individuals exposed to bancroftian filarial infection by sandwich-ELISA.
Demerits of polyclonal antibodies in detecting the antigens (infection)

Polyclonal antibodies raised for developing an immunodiagnostic technique for epidemiological studies have certain drawbacks such as insufficient specificity because of the several common or cross-reactive antigens between the other helminth and human filarial parasites. This is also because they are mixture of several antibodies produced by the animal in which they were raised. Hence, there is a need to raise filarial antigen specific antibodies and the alternate would be development of monoclonal antibodies (MAbs) to overcome this problem as MAbs are the appropriate reagents and are specific in binding, homogenous and can be produced in unlimited quantities (Harlow & Lane, 1988)

Monoclonal antibodies

Köhler and Milstein (1975) developed a technique that allows the growth of clonal populations of cells secreting antibodies with a defined specificity. In this technique an antibody – secreting cell, isolated from an immunized animal, is fused with a myeloma cell, a type of B-cell tumor. These hybrid cells or hybromas can be maintained in vitro and will continue to secrete antibodies with a defined specificity. Antibodies that are produced by hybridomas are known as MAbs (Harlow & Lane, 1988). They are of immense value and interest because the method of their production allows for the
manufacture of endless quantities of a single antibody (Goding, 1983). The production of MAbs in large quantities opened the door for many advances made in our understanding of the basic polarization of the working of the immune system. The application of this hybridoma technology has a great potential in immunoparasitology to produce MAbs of interest on diagnosis and immuno prophylaxis (Reddy et al., 1984). Hence, recent efforts in immunodiagnosis of filariasis have focused on the establishment of MAbs.

Monoclonal antibody based antigen detection assays

MAbs have been developed in the past either against somatic or e/s products of filarial parasites. I3 B4 & I5 D6 are MAbs produced against the antigenic epitopes common between the bovine Setaria cervi and human filarial parasites. I3 B4 based sandwich-ELISA could detect circulating antigens in microfilaremic cases (Kaushal & Kaushal, 1995). Forsyth et al. (1985) used Gib 13 MAb raised against Onchocerca gibsoni for antigen detection in sera and urine samples of microfilaraemic cases. By using two site IRMA Reddy et al. (1984) found WBF 34 MAb raised against W. bancrofti mf e/s antigen was useful to detect circulating filarial antigen. Lal et al. (1987) raised phosphoryl choline (pc) reactive MAbs against a 200 kDa circulating Ag of bancroftian filarial cases & in ELISA those antibodies could detect filarial antigen in 93% of mf patients and 46% of chronic cases.
Weil *et al.* (1987) raised another set of MAbs, AD 12.1 & DH 6.5, which detected 98% microfilaraemics and 16% amicrofilaraemics patients. AD 12.1 recognized a 200 kDA antigen in sera of filarial patients (Weil & Liftis, 1987). Og4C3 another MAb raised against *O. gibsoni* adult antigen by More & Copeman (1990) could detect antigen in microfilaraemic as well as amicrofilaraemic cases.

### 2.8 Current status of filarial immunodiagnosis

Diagnostic tests are available now commercially for the detection and differentiation of active infection which eventually are expected to replace the night blood examination of parasitological diagnosis. As stated above More & Copeman (1990) raised MAb Og4C3 against *O. gibsoni* adult antigen which were found to detect antigen. It is now commercially available as an ELISA kit (TropBio Pvt. Ltd, Australia) to detect circulating *W. bancrofti* antigens in an individual. Added to this, individual immunochromatographic test cards (ICT diagnostics, Balgowlah, Australia) coated with AD12.1 monoclonal antibody (mentioned above) against *W. bancrofti* to detect CFA has been widely used. When these diagnostic kits were evaluated by different laboratories the sensitivity ranging from 72-100% have been reported for the commercially available kits viz., ICT card test and Og4C3 ELISA kit respectively (Pani *et al.*, 2000; Rocha *et al.*, 1996). The specificity of these assays has been found to range from
98.4-100%. A sensitivity of 50.3% has also been reported for filter paper format of Og4C3 ELISA test (Gyapong et al., 1998). Also, recently false positivity of the ICT card-test if not read exactly at 10 minutes has been reported which is difficult to follow in the field conditions to cover few thousands of the patients (Rajgor et al., 2002). However these tests are expensive, need to be imported and hence majority of the people in developing countries like India cannot afford to use them. Therefore, there is a need to develop indigenous immunotechniques for the diagnosis of filariasis, as the effective control of filariasis depends on early and specific diagnosis of filariasis.

2.9 Molecular tools

Use of recombinant antigens

New antibody tests based on recombinant antigens are reasonably sensitive and more specific than earlier tests based on native filarial antigens. Recombinant filarial antigen SXP 1 has been explored in the diagnosis of lymphatic filariasis (Dissanayake et al., 1994). Using this SXP 1, filarial antibodies were detected in 80% of microfilaraemic cases but only in 33% of acute filarial cases / cases with indirect evidence of filarial infection. Two recombinant clones of B. malayi antigens Bm M 5 and Bm M14 were developed. Assays based on these two antigens to measure IgG4 abs were useful to detect 90% of lymphatic filarial sera from Indian and Egyptian patients.
DNA Probes

New methods are being developed using DNA probes to identify filarial larvae in body fluids (Poole & Williams, 1990) and mosquito vector (Carlow et al., 1987). Molecular approaches, such as polymerase chain reaction (PCR) assays, promise high specificity and sensitivity and rapidity in the diagnosis of infections. PCR assay based on Hha I repeat sequences has been developed and are being used to detect B. malayi infection (Williams et al., 1988; Lizotte et al., 1994). The above stated assay has been reported to have high specificity and sensitivity over other assays developed for W.bancrofti (Chandrashekar, 1997). DNA probes along with PCR amplification are most sensitive and reliable to detect filarial parasites.

2.10 Other diagnostic tools

Ultrasonography

Non-invasive techniques for detecting filarial parasites in situ have been used for diagnosis. Ultrasonography has been used to detect O. volvulus adult parasites in subcutaneous nodules (Amaral et al., 1994). Ultrasound technique has also been used to detect adult W. bancrofti in infected male patients by imaging scrotal region (Dreyer et al., 1994).
Lymphangioscintigraphy

To investigate lymphatic abnormalities caused by filarial infection, lymphangioscintigraphy of patients has been carried out by Witte et al. (1993) with success. Lymphangioscintigraphy is a safe non-invasive method to examine peripheral lymphatic system including tunical and nodal abnormalities, but this technique is mainly aimed at the management of chronic cases.

Magnetic Resonance Imaging

Magnetic Resonance Imaging to detect parasites and lymphatic abnormalities may also be useful in filarial infection (Thompson, 1991). This method has been tried by Case et al. (1992) to observe lymphatic abnormalities in ferrets infected with B. malayi.

2.11 Prevention and control of filariasis

2.11.1 Chemotherapy

The primary goal of treatment is to eliminate mf from the blood of infected individual so that transmission of the infection by the mosquito can be interrupted. Secondly treating the individual for complete cure of infection.

i) Diethylcarbamazine (DEC)

Methyl 1,4-diethylcarabamoyl piperzine (DEC), a piperazine derivative (Hewitt et al., 1947, 1948) is still considered the drug of
choice for treating or suppressing lymphatic and other filarial infections (Mak et al., 1991). It is a powerful microfilaricidal agent and is rapidly absorbed from gastro-intestinal tract.

Dose regimen (Pani et al., 1997)

For treating the individual with mf in the circulation

The two regimens, which are widely accepted and are in use are

a. 6 mg/kg body weight per day in 3 divided doses for 12 days (standard regimen followed)

b. 6 mg/kg body weight in a single dose given at monthly interval for 12 months Regimen two of the above gives better results.

ii) Ivermectin

A single dose of Ivermectin 400 μg/ kg of body weight to be repeated at 3 monthly intervals for one year (4 doses a year).

iii) Ivermectin and DEC

A single dose of Ivermectin 200 μg and DEC 6 mg/kg of body weight, which may be repeated after 6 months.

Or

A single dose of DEC 6 mg/kg of body weight and Albendazole 600mg (for adults) is found to be effective. This may be repeated after one year, after a re-examination of blood.
2.11.2 Vector control

This can be achieved by either the use of insecticides or by the use of biocontrol agents.

2.11.3 Reduction of man-vector contact

By the use of nets, repellents, personal protection measures etc.

2.11.4 Health education

For the community to be aware of early signs and symptoms of the disease.