Introduction
Filariasis: Present Scenario

Filarial nematodes belonging to superfamily Filarioidea infect more than 150 million humans worldwide and cause the dreadful disease Filariasis. It threatens one billion individuals in Africa, India, South East Asia and the America. Filarial diseases are still far from being vanished, thus pose a challenge for multiple areas of research (Hoerauf et al., 2001).

Filariasis has been identified as a potentially eradicable disease and the 50th World Health Assembly passed a resolution that ‘elimination of filariasis as a public health problem’ should be considered a priority by member states. A ‘global alliance’ consisting of the World Health Organization (WHO), the World Bank, UNICEF, the Carter Centre, Glaxo Smith Kline, Merck and Co., academic institutions, and other non-government agencies has been formed to help facilitate the task (WHO, 1997).

Filariasis has been categorised into lymphatic, subcutaneous and serous cavity on the basis of the pathological symptoms. Under each category is a list of the filarial nematode species that cause the associated conditions (Table 1). Lymphatic filariasis is an infection of the human lymphatic system by filarial nematodes. It is classified into two groups viz. Bancroftian (caused by W. bancrofti) and Malayan (caused by B. malayi and B. timori). W. bancrofti is responsible for 90% of lymphatic filarial cases (Melrose, 2002). Lymphatic filariasis has been identified by the WHO as the second leading cause of permanent and long term disability worldwide. It infects 120 million people or 2.0% of the world’s population and continues to be a worsening problem, especially in Africa and on the Indian subcontinent. India alone contributes about 47% of the global prevalence of chronic patients and 39% of the population at risk of infection (WHO, 1997). Elephantiasis (Fig. 1), lymphoedema and genital pathology afflict 44 million people; another 76 million have parasites in their blood and hidden internal damage to their lymphatic and renal system (Ottesen et al., 1997). While acute episodic adenolymphangitis causes severe physical suffering, chronic disease such as lymphoedema and hydrocele causes permanent disfiguration and psycho-social problems (Pani et al., 1995; Gyapong et al., 1996;
<table>
<thead>
<tr>
<th>Parasite</th>
<th>Location in body</th>
<th>Principal vector</th>
<th>Major Pathology</th>
<th>Geographical Distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>I. Lymphatic Filariasis</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wuchereria bancrofti</td>
<td>Lymphatics</td>
<td>Blood</td>
<td>Culex quinquefasciatus</td>
<td>Lymphangitis, Elephantiasis</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Tropical and subtropical countries</td>
</tr>
<tr>
<td>Brugia malayi</td>
<td>Lymphatics</td>
<td>Blood</td>
<td>Mansonia spp</td>
<td>Lymphangitis, Elephantiasis</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Southeast Asia</td>
</tr>
<tr>
<td>Brugia timori</td>
<td>Lymphatics</td>
<td>Blood</td>
<td>Anopheles barbirostris</td>
<td>Adinolymphangitis</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Timor and some other islands of</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>eastern Indonesia</td>
</tr>
<tr>
<td><strong>II. Subcutaneous filariasis</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Loa loa</td>
<td>Connective tissue, Conjunctiva</td>
<td>Blood</td>
<td>Chrysops spp</td>
<td>Transient swelling, Temporary loss of vision</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Tropical Africa</td>
</tr>
<tr>
<td>Onchocerca volvulus</td>
<td>Subcutaneous nodules</td>
<td>Skin, eyes</td>
<td>Simulium spp</td>
<td>Loss of vision, Dermatitis</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Africa, Mexico, Guatemala, Foci in Central and South America</td>
</tr>
<tr>
<td>Mansonella streptocerca</td>
<td>Subcutaneous</td>
<td>Skin</td>
<td>Culicoides</td>
<td>Dermatitis</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>West Africa</td>
</tr>
<tr>
<td><strong>III. Serous cavity filariasis</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mansonella ozzardi</td>
<td>Peritoneum and Pleura</td>
<td>Blood</td>
<td>Culicoides</td>
<td>Not well defined</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>West Indies, Central and South America</td>
</tr>
<tr>
<td>Mansonella perstans</td>
<td>Peritoneum and Pleura</td>
<td>Blood</td>
<td>Culicoides</td>
<td>Not well defined</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Africa and South America</td>
</tr>
</tbody>
</table>
Filariasis: Present Scenario

Pani and Srividya, 1995; Ramaiah et al., 1996a). It results in loss of work, productivity, direct and indirect economic loss and functional impairment (Pani et al., 1995; Ramaiah et al., 1996a b; 1997a b; Ramu et al., 1996). Consequently, the disease is a significant impediment to socio-economic progress of the endemic countries.

Fig. 1 Lymphatic filariasis: Elephantiasis is the last consequence of the swelling of limbs

Subcutaneous filariasis infects the areas beneath the skin and whites of the eye. *O. volvulus* is the causative agent of river blindness or onchocerciasis. It affects 18 million individuals living in Sub-Saharan Africa and in isolated pockets of Central and South America. Approximately 500,000 people suffer significant visual loss, of whom approximately 50% are blind (WHO, 1995; WHO, 2000). The major complication of onchocerciasis is the development of lesions in the eye which include sclerosing keratitis, secondary glaucoma and cataract, coroidoretinitis and fluffy corneal opacities. It also causes atrophy of the skin resulting in loss of elasticity. The infection of African eye worm *L. loa* is characterized by ‘calabar swellings’ which are inflammations found anywhere in the body but most commonly in the extremities. Serious complications such as cardiomyopathy, encephalopathy, nephropathy and pleural effusion have been recorded. Serous cavity filariasis on the other hand infects body cavities but does not cause disease.

Occult filariasis is the term applied to clinical conditions not directly due to lymphatic involvement, but to hypersensitivity reactions to filarial antigens. The best studied syndrome of occult filariasis is Tropical Pulmonary Eosinophilia, which presents with low grade fever, loss of weight, anorexia and pulmonary symptoms such
Filariasis: Present Scenario

as dry nocturnal cough, dyspnoea and asthmatic wheezing. Blood eosinophil count is above 3000 per cmm and may even go up to 50,000 or more. Histological findings during early weeks of clinical symptoms show histocytes, infiltration of alveolar spaces and interstitium followed by bronchopneumonia and eosinophilic abscesses (Ottesen, 1984). After several months the infiltrates characterized by eosinophils, histiocytes and lymphocytes often organise into granulomatous responses. Eosinophilic granulomatous reactions have been found in lung, liver, lymph nodes and spleen of affected individuals.

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 dioecious adult worms. After mating the females release large numbers of mf which continue the cycle when ingested in the blood meal (Fig. 2).

Fig. 2 Generalized Life cycle of filarial worm

Each of the major life cycle stages (mf, L3 and adults) has unique immunological characteristics. This disease is due to host’s immune response to the worms, particularly dying worms. Pattern and severity of the disease varies with the
site and stage of species. The infections are chronic and worst in individuals constantly exposed to reinfection.

Traditionally, diagnosis of lymphatic filariasis has depended upon the detection of mf in blood collected around midnight in areas where mf exhibit nocturnal periodicity, and around midday where periodicity is diurnal. These methods include stained film, membrane filter, concentration and Knott’s tests. Diagnosis has been revolutionized by the introduction of filarial antigen tests, which do not depend on the presence of mf, removing the need to take night bloods. One of the tests, the ICT test, can be done by finger-stick in the field and the results are available almost immediately. PCR methods have been successfully used for the detection of filarial DNA in blood and by ultrasonography adult worms can be directly detected in tissues (Melrose, 2002).

No vaccine is available at present to prevent filariasis (Grieve et al., 1995; McCarthy and Nutman, 1996). Vector control on the other hand is a time-taking endeavor and is rendered less effective due to the development of insecticide resistance (Schuurkamp et al., 1987). Drug administration is the major line of action in filariasis control, but in spite of decades of efforts, no single therapeutic agent that could effectively combat the disease by eliminating the adult worms and that is devoid of adverse side reactions could be developed. Currently, DEC and Ivermectin are the two drugs of choice but both are only microfilaricidal and far from ideal. DEC has adverse side reactions (Fan, 1992) and cannot be recommended in areas where there is co-existing onchocerciasis or loiasis (Ottesen and Ramachandran, 1995). Furthermore there are reports of parasites acquiring resistance to ivermectin (Xu, et al., 1998). Efficacy of the control of filariasis thus remains disappointing due to lack of appropriate one-shot chemotherapeutic agents capable of eliminating adult parasites (Ottesen and Ramchandran, 1995). Adult worms live in the body of the host over years due to which filariasis keeps on relapsing. Thus in view of current scenario, an adulticidal agent is absolutely needed to destroy the adult worms, which are being implicated as major cause of development of the disease manifestations (Ottesen, 1992; Dreyer et al., 1995).
Glutathione: A brief overview

Glutathione (γ-glutamyl cysteinyl glycine), a ubiquitous tripeptide (Fig. 3) found in almost all organisms has been the subject of much research since its discovery by de Rey Pailhade (1888a, b), and especially after its structural elucidation about a half century later by Hopkins and others in 1930. Glutathione exists in the thiol-reduced (GSH) and disulfide-oxidized (GSSG) forms (de Leve and Kaplowitz, 1991). Eukaryotic cells have three major reservoirs of GSH. Almost 90% of cellular GSH are in the cytosol, 10% in the mitochondria, and a small percentage in the endoplasmic reticulum. In the endoplasmic reticulum, where GSH is implicated in protein disulfide bond formation, the GSH to GSSG ratio is 3:1. In the cytoplasm and mitochondria ratio exceed from 10:1 to 100:1 depending upon the type and state of cell (Meredith and Reed, 1982; Hwang et al., 1992). Cytosolic GSH has the half life of 2-4 h whereas mitochondrial GSH has the half life of about 30 h (Meister, 1995). GSH is found in the concentration range of 1-10 mM in virtually all mammalian cells (Larsson et al., 1983).

Fig. 3 The structure of reduced glutathione

The γ-glutamyl linkage promotes intracellular stability and the sulfhydryl group is required for GSH’s functions. The peptide bond linking the amino-terminal glutamate and the cysteine residue of GSH is through the γ-carboxyl group of glutamate rather than the conventional α-carboxyl group. This unusual arrangement
resists degradation by intracellular peptidases and is subject to hydrolysis by only one known enzyme, γ-glutamyl transpeptidase (γ-GT). Furthermore, the carboxyl terminal glycine moiety of GSH protects the molecule against cleavage by intracellular γ-glutamyl cyclotransferase (γ-GCT) (Meister, 1988a). As a consequence GSH resists intracellular degradation and is only metabolised extracellularly.

The three most important redox systems we commonly find within the cells are nicotinamide adenine dinucleotide phosphate (NADPH/ NADP⁺), thioredoxin (TRX_red/ TRX_ox) and glutathione (GSH/ GSSG). Among these, glutathione is the most important since glutathione concentration is about 500-1000 fold higher than TRX and NADPH, so changes in reduced/oxidised glutathione buffer directly reflect intracellular redox alterations (Schafer and Buettner, 2001). Besides its function as intracellular redox buffer, glutathione possesses several other roles. It can function as modulator of activity of thiol-dependent enzymes, which possess critical cysteine residues sensitive to redox changes (Finkel, 2000; Klatt and Lames, 2000). GSH can also represent a cofactor for antioxidant enzymes such as GSH-peroxidase (GPX), a selenium containing enzyme involved in the reduction of many peroxides, especially membrane lipid peroxides formed upon oxidative insults (Flohe, 1978):

\[ \text{H}_2\text{O}_2 + 2\text{GSH} \rightarrow 2\text{H}_2\text{O} + \text{GSSG} \]
\[ \text{R}_0\text{O}_2\text{H} + 2\text{GSH} \rightarrow 2\text{ROH} + \text{GSSG} \]

Although GSH does not react nonenzymatically with H₂O₂, another function for GSH in antioxidant defence that depends upon its ability to react with carbon centred radicals (R*) has been proposed by Winterbourn (1993) in “free radical sink” hypothesis:

\[ \text{R}^* + \text{GSH} \rightarrow \text{RH} + \text{GS}^* \]
\[ \text{GS}^* + \text{GS}^- \rightarrow \text{GSSG}^{*^-} \]
\[ \text{GSSG}^{*^-} + \text{O}_2 \rightarrow \text{GSSG} + \text{O}_2^{*^-} \]
\[ 2\text{O}_2^{*^-} + 2\text{H}^+ \rightarrow \text{H}_2\text{O}_2 + \text{O}_2 \] (Superoxide Dismutase)

Mitochondria do not synthesize glutathione; rather they obtain their GSH from cytosol (Griffith and Meister, 1985). While the electron transport system is highly
efficient, some ROS 'leak.' Since mitochondria do not contain catalase, they depend upon GPXs and non-enzymatic reaction with GSH to protect against ROS toxicity.

Detoxification of xenobiotics or their metabolites is another major function of GSH. These compounds are electrophiles and form conjugates with GSH (GSH-S-conjugates) either spontaneously or enzymatically in reactions catalysed by GSH-S-transferases (GSTs) (Kretzschmar and Klinger, 1990). GSTs catalyses the conjugation of the GSH thiolate anion with a multitude of exogenously and endogenously derived toxic compounds including the secondary products of lipid peroxidation (Mannervik, 1985; Ketterer et al., 1988). The conjugates formed are usually excreted from the cell.

A further role for GSH has been suggested in intracellular copper transport and detoxification. In fact, through the cysteine thiol group it can bind Cu^{2+} contributing to copper delivery to the apoprotein of copper enzymes (Ciriolo et al., 1990; Ferreira et al., 1993). Moreover, in doing so, GSH decreases the dangerous effect of free intracellular copper by impeding potentially toxic redox reactions among metals and oxygen. GSH is also concerned directly or indirectly with important biological phenomenon such as synthesis of DNA and proteins, microtubular related processes, and immune phenomenon. Finally, GSH may represent storage for cysteine, which can be toxic when present at high concentration (Meister, 1988b) (Fig. 4).

Cellular GSH levels reflect a steady state balance between synthesis, salvage and loss. Synthesis includes de novo formation of GSH from the constituent amino acids. De novo GSH biosynthesis involves two step reaction catalysed by the sequential actions of regulatory Glutamate cysteine ligase (GCL) and Glutathione synthetase (GS), respectively:

\[
\begin{align*}
\text{L-Glutamate} + \text{L-Cysteine} + \text{ATP} & \rightarrow \text{L-} \gamma \text{-Glutamyl-L-cysteine} + \text{ADP} + \text{Pi} \\
\text{L-} \gamma \text{-Glutamyl-L-cysteine} + \text{Glycine} + \text{ATP} & \rightarrow \text{GSH} + \text{ADP} + \text{Pi}
\end{align*}
\]

Salvage of GSH involves GSH-reductase (GR) mediated regeneration of GSH from GSSG and the direct synthesis of GSH from L-\gamma-glutamyl-L-cysteine and glycine, bypassing the first step of de novo. GR normally maintains the total glutathione pool in a predominantly reduced state and catalyses NADPH dependent reaction as follows (Akerboom et al., 1982; Kehrer and Lund, 1994):

\[
\text{GSSG} + \text{NADPH} \rightarrow \text{GSH} + \text{NADP}^+
\]
**Fig. 4 Functions of Glutathione (GSH)**

- **Protein biosynthesis**
  - Act as reservoir for cysteine

- **Maintaining other antioxidants in reduced state**
  - Ascorbate
  - α-Tocopherol

- **Detoxification/Xenobiotic Metabolism**
  - Catalysed by Glutathione-S transferases

- **Enzyme linked Antioxidant function**
  - Enzymes involved are
    - Glutathione peroxidases
    - Glutathione reductase
    - Glutathione-S-transferases

- **Direct non enzymatic Free radical scavenging**

- **Coenzyme functions**
  - GSH act as coenzyme to
    - Glyoxylase
    - Prostaglandin peroxidase
    - Formaldehyde dehydrogenase etc.

- **DNA biosynthesis**
  - Reduction of ribonucleotides to deoxyribonucleotide precursors of DNA

- **Maintenance of essential thiol status of proteins**

- **Others**
  - Modulating
    - Microtubular function
    - Immune functions
    - Leukotriene metabolism
    - Thyroid hormone metabolism
    - Melanin formation
    - Neurotransmission
The breakdown of GSH occurs extracellularly and is catalysed by γ-GT and dipeptidases bound to the external surfaces of cell membranes. GSH is exported to the membrane bound enzymes under normal physiological conditions. Small amounts of GSSG may be transported normally, and such export increases markedly when the intracellular GSSG level is increased. Breakdown of GSH S-conjugates also requires export to the membrane bound enzymes. Thus γ-GT acts on exported GSH, GSSG, and GSH S-conjugates. Transpeptidation by γ-GT which takes place in the presence of amino acids and GSH, leads to formation of γ-glutamyl amino acids and cysteinylglycine. The most active amino acid acceptor is cystine. γ-Glutamyl amino acids formed in this way are transported into the cell and are substrates of the intracellular enzyme γ-GCT, which converts γ-glutamyl amino acids into 5-oxoproline and free amino acids. 5-oxoproline is converted to glutamate in the ATP dependent reaction catalysed by 5-oxoprolinase.

Interaction of γ-GT with GSH and cystine leads to the formation of γ-glutamylcystine, which is transported and reduced intracellularly to form cysteine and γ-glutamylcysteine. Utilization of the latter by GS completes an alternative pathway which bypasses the step catalysed by GCL and appears to serve as a recovery system for cysteine moieties. Cysteinylglycine splits on the membrane by cysteinylglycine dipeptidase to form cysteine and glycine to be again utilised intracellularly for GSH biosynthesis (Meister, 1991).

Glutathione homeostasis can be perturbed in a number of ways. The most frequently encountered alterations of normal steady state are:

(1) Increased consumption of GSH secondary to exposure to or generation of one or more electrophilic species, including oxidants and some alkylating intermediates.

(2) Decreased availability of amino acid precursors, usually cysteine.

(3) Diminished activities of GCL, the rate limiting enzyme in GSH biosynthesis. Inhibition of GCL can be accomplished by administration of L-buthionine-S-sulfoximine (BSO).
(4) Decreased ATP availability. Although GCL and GS are ATP dependent, as are other transformations in GSH homeostasis, the impact of ATP limitations on GSH metabolism has received less attention.

(5) Increased cellular export of GSH, or more often GSSG

Studies on the enzymology of GSH metabolism have been facilitated by the series of selective enzyme inhibitors. Of the major importance has been the development of selective enzyme inhibitors which are potential therapeutic agents. It is now possible to increase or to decrease cellular GSH levels and also to selectively inhibit reactions involved in GSH metabolism. Such modulation of GSH metabolism has therapeutic promise in the selective destruction of cells and in their protection. Previously, attempts have been made to target the glutathione metabolism of certain parasites and tumour cells on the basis of the fact that normal non cancerous and host cells have a considerable excess of glutathione as compared to tumours and parasitic cells which have GSH concentrations close to that necessary for survival (Meister, 1983), an equivalent effect of GSH depletors on the GSH levels of normal and target tissues would be expected to favour survival of normal cells. Parasites are more sensitive to oxidative stress as they have to cope with the reactive oxygen species of the host along with their own oxidative strain for their continued survival. In the present thesis attempts have been done to explore some aspects of filarial glutathione metabolism and to target it to get some antifilarial leads.