

ABSTRACT

Human lymphatic filariasis is caused mainly by infection with nematode parasite *Wuchereria bancrofti* and *Brugia malayi* affecting about 128 million people worldwide. The current method of diagnosis by microscopic examination of microfilariae (mf) in night-blood samples is insensitive and tedious. Hence there is a need to develop sensitive and less tedious diagnostic tests, that can detect early stages of infection. Although antibody based diagnostic methods are available, circulating filarial antigen detection is a better choice to detect active filarial infection.

As part of this work the presence of circulating filarial antigen (CFA) was studied in individuals living in an area endemic for *W. bancrofti* infection in Chennai, India using a commercially available Og4C3 monoclonal antibody-based ELISA. All microfilaraemics (MF) and 20% of endemic normals were positive but none of the chronic pathology (CP) showed the presence of CFA by Og4C3 assay. Hence this test could be used to study the individuals who are having active and prepatent filarial infection. The CFA levels in day and night blood samples were same, suggesting that the assay can be performed during the daytime. For large-scale field studies the CFA levels were measured in samples (20µl blood) collected on filter strips and from serum of the same patients. The levels of CFA was same using either the filter paper eluate or serum samples. In order to study the utility of the assay for therapeutic follow up of different DEC treatment, the CFA levels were measured in MF patients before and after treatment.

A promising diagnostic candidate recombinant antigen gene SXP from *B. malayi* adult cDNA library was expressed in T7 expression vector (pRSETB). The recombinant protein from the clone pRBSXP was purified using IMAC column and monospecific antibodies raised in mice and rabbit. Further using the purified recombinant antigen pRBSXP, an IgG4 specific ELISA was developed and it was observed that high levels of pRBSXP specific IgG4 was present in the microfilaraemics (MF) individuals with either bancroftian or brugian infection. Moreover the recombinant antigen specific antibodies were detected in blood collected on filter strips by finger prick, making it applicable for the field studies.

The changes in the pRBSXP-specific IgG4 and the CFA (Og4C3) levels were monitored in MF patients who were treated with different doses of DEC to study the changes that take place following chemotherapy. It was observed that either single or the regular 12 days dose of DEC could make significant drop in the CFA levels by day 14 with a significant raise in the IgG4 levels ($p < 0.05$). By one month, the CFA levels increase and remains high upto 3 months with a drop in the IgG4 levels, this could be because of the adult worms might be killed or injured as reported earlier. Thus there is a drop in CFA release from the adult worm which is quantitated using Og4C3 assay, indirectly indicates the adult worm burden. Hence the recombinant antigen specific IgG4 in MF patients can be used as an additional diagnostic tool along with CFA assay to monitor the post-therapeutic effects of various drugs and also the efficacy of various control programs.

In the next part of the thesis an attempt was made to develop ELISA for the identification of CFA using antibody to recombinant filarial antigen. Using the purified pRBSXP antigen monospecific antibodies were raised in mice and rabbits and an antigen detection assay developed to detect individuals with active filarial infection. The results of this assay is promising and it was observed that the antigen assay was able to detect both bancroftian or brugian filariasis. Thus it is advantageous over the existing Og4C3 assay which is specific for bancroftain filariasis.