IMMUNODIAGNOSIS OF HUMAN LYMPHATIC FILARIASIS: A REVIEW

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ABSTRACT

A monoclonal antibody-based antigen detection system was used to detect the levels of circulating antigen in filarial patients before and after treatment with DEC and in normal individuals living in an area endemic for *W.bancrofti* infection in Chennai, India. The present study was to show the use of this assay as a means of efficient screening for filariasis in an endemic area where blood was absorbed onto the filter paper by finger prick during day time. The results of the antigen levels collected onto filter strips correlated with their corresponding plasma antigen levels ($r=0.83$). In microfilaraemics, DEC treatment did not alter the levels of circulating antigens upto a period of one month. We conclude that this monoclonal antibody based ELISA using filter strips may be used in daytime and can replace the existing routine night blood survey.

KEY WORDS : Antigen, ELISA, filariasis, diagnosis.

INTRODUCTION

The filarial parasites *Wuchereria bancrofti* and *Brugia malayi* are the major cause of human lymphatic filariasis in the tropics and subtropical regions of the world with an estimated 120 million people infected and an additional 900 million people at the risk of infection (1). The disease exhibits distinct clinical stages that include microfilaraemics (MF) who are asymptomatic and microfilaraemic, chronic pathology (CP) who are symptomatic and amicrofilaraemic, endemic normals (EN) who are asymptomatic and amicrofilaraemic, and a rare group of patients with chronic lung disorder that leads to the tropical pulmonary eosinophilia (TPE) caused by immunologic hyperresponsiveness to the filarial parasite. The diagnosis of filarial infections can be broadly classified based on various detection systems. Parasitological diagnosis is the detection of parasite microfilariae (mf) by the use of thick blood film, counting chamber, Knott's concentration technique and membrane filtration tech-

niques. Lymphatic imaging by lymphangiography or lymphoscintigraphy has a potential advantage in the early evaluation of lymphatic damage in MFs. Recent efforts in immunodiagnosis have focused on the establishment of specific immunological reagents such as monoclonal antibodies and recombinant antigens for the early detection of filarial infections in endemic areas. The routine ways of diagnosing active filarial infections by detecting the presence of microfilaria in the night blood smears is less sensitive because of the nocturnal periodicity of the microfilaria. Further there is a possibility of missing microfilaria in the blood smear if they are present in extremely low numbers and thereby introducing an error in diagnosing the active infection.

MATERIALS AND METHODS

Antibody Detection in Human Lymphatic Filariasis

A relatively better method for the detection of active infections using antibody based enzyme linked immunosorbent assay (ELISA) has been developed by several laboratories all over the world as well as in our laboratory (2). Filarial

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parasite specific antibody detection assays are of little practical value in endemic areas because seropositivity to this test simply indicates that the individuals have been sensitized to parasite antigens and cannot be interpreted as evidence of active infection. Further, antibody detection assays are unsatisfactory due to the fact that these antibodies cross-react with other helminth infections and cannot reliably distinguish between past and current infection. Serodiagnostic tests for lymphatic filariasis have been substantially improved either through the use of excretory-secretory (ES) antigens (3) which appear to be subsets of whole worm somatic extracts or through the use of specific reagents to detect filarial parasite specific antibody isotypes or subclasses (4). It is also expected that the availability of monoclonal antibodies selected for stage and species-specific molecularly defined filarial antigens will permit the rapid development of new serodiagnostic assays for lymphatic filariasis.

Antigen Detection in Human Lymphatic Filariasis

Monoclonal antibody based ELISAs have been developed by a number of laboratories all over the world (5,6) to detect and quantify filarial specific antigens in sera, urine and other body fluids of persons with lymphatic filariasis. these assays filarial antigens are sensitive and specific as seropositivity to this test reflects the presence of filarial worms. Thus antigen detection assays which are capable of detecting all individuals with active infection and persons with sub clinical infections in an endemic area, have an advantage over traditional diagnostic techniques. The development of stage and species specific recombinant antigens from genomic and cDNA libraries of filarial parasites, which is underway, appears to have great potential in their use for serodiagnosis in human lymphatic filariasis.

PCR Based Detection System in Human Lymphatic Filariasis

A polymerase Chain reaction (PCR) assay based on highly repeated DNA has been developed for the diagnosis of *W.bancrofti* infection (7). Studies have been shown that this assay is highly sensitive and can even detect single microfilaria in the blood samples from infected individuals. Though this technique is the best compared to other methods, it has its own disadvantage in that it can be performed only by skilled personnel and requires sophisticated instrument. Further the cost is probably one of the factors in determining its widespread use in the field conditions.

In the present study, we have used commercially available monoclonal antibody (Og4C3) based sandwich ELISA kit (Tropical Biomedical Institute, Queensland University, Australia) for detecting circulating filarial antigens as a means of primary surveillance of filariasis in an endemic area in Chennai, India. The circulating antigen levels in MF individuals before and during treatment with Diethylcarbamazine (DEC) were also monitored. Further this technique has been simplified to suit the field conditions where capillary blood was collected onto filter strips to quantitate the circulating antigen levels. More importantly, antigen based ELISA systems have the advantage of estimating the circulating antigen levels in the plasma samples collected either in the day or night whereas the routine parasitological test requires night blood samples.

RESULTS AND DISCUSSION

In the present study we have used Og4C3 monoclonal antibody based ELISA for detecting circulating antigen in filarial patients. It is specific in that only MF individuals were positive and all the CP were negative. Plasma from non-endemic

Table 1 : The antigen units and microfilaria count in different clinical groups of filariasis patients.

Clinical category	Positive by ELISA number (Ag units range)	Negative by ELISA number (Ag units range)	Mean mf count (mf/ml range)
Asymptomatic microfilaraemic (MF) n=40	40 (51- 2560)	0 (0)	252 (4-1000)
Chronic lymphatic obstruction (CP) n=10	0 (0)	10 (0)	0 (0)
Endemic normals (EN) n=10	2 (449,558)	8 (0)	0 (0)
Non endemic normals (NEN) n=10	0 (0)	10 (0)	0 (0)

normals (NEN) used as controls, also showed negative by Og4C3 (table 1). Hence this ELISA can be used for the early diagnosis of filariasis. Two of the ten EN individuals (i.e. 20%) are positive by this test but negative by night blood smear suggesting that this test can be used for identifying unisexual filarial worm infection or prepatent stage of infection. Chanteau et. al. (8) have demonstrated that the antigen detected by Og4C3 is a possible indicator of adult worm burden and that it is likely that this test can be used in immunomonitoring of filariasis. Lymphoproliferation and cytokine response studies done on different clinical groups of filarial patients have shown that MF individuals have suppressed Th1 response to filarial specific antigens as compared to CP and EN individuals (9). In addition, the CP individuals have a mixed type of cytokine response (both Th1 and Th2) and were able to clear the antigen by the cell-mediated immune response and so these individuals are antigen negative by the monoclonal antibody based assay.

We compared the circulating antigen levels in the plasma and capillary blood collected from the same individuals by finger-prick and we found a positive correlation ($r = 0.835$) between them (Fig. 1). The collection of blood in filter strips at

any time of the day is of importance in seroepidemiological surveys of filariasis where preservation of plasma is a problem and this makes serological screening less feasible for community based programmes. Hence this test can possibly replace the existing survey by night blood smear which is sometimes difficult to perform. Moreover the advantage of collecting blood by finger-prick is that it requires minimum facilities under field conditions and is less troublesome than the more invasive venipuncture procedure. Samples collected on the filter paper are dry, light, easy to handle and can be transported.

The monoclonal antibody based ELISA has been used for monitoring the levels of the circulating antigen in MF patients following DEC treatment. Although the mf level is zero by the seventh day, it is seen that antigenemia does not change significantly and remains high upto a month after treatment. (Fig 2) This, therefore, suggests that neither the presence of mf nor the products released by them due to their killing by DEC seems to have an effect on circulating antigen levels. Evidently, our results are in parallel with earlier findings where the levels of circulating antigen were monitored for a period of 18 months in endemic areas of other parts of the world and it

Fig 1 : Comparison of filarial specific antigen levels in the plasma and the blood absorbed onto the filter strips in the same patient. Results expressed as Mean $\pm$ SEM of 40 patients.

Fig 2 : Correlation of filarial specific antigen levels and microfilaria count in the microfilaraemic patients before and after treatment with Diethyl Carbamazine (DEC). Result expressed as mean antigen units and microfilaria count/ml of nine patients.

remained unaltered. This suggests that Og4C3 antibody appears to recognize a particular antigen released by the adult worm (8). Currently such long term follow up of the MF patients using Og4C3 monoclonal antibody based ELISA is in progress in our laboratory.

In conclusion, this monoclonal antibody based kit for detecting circulating filarial antigen levels provides a sensitive, specific, and practical method for detection of MF cases in endemic areas. The very fact that the assay can be performed using day blood samples collected on

filter strips maximises the convenience and hence this method of diagnosis appears to be the first step in the control of spread of filarial infections. Moreover, since the antigen levels do not correlate with the mf density, it will be possible to study the prognosis of a patient with active infection by monitoring the antigenemia for a period of one year or more. This is essential in an endemic area for arresting transmission and prevention of pathology associated with clinical manifestations rather than looking at the mf density by conventional methods.

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