Seroprevalence of lymphatic filariasis at Puducherry

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Abstract Lymphatic filariasis is a major vector borne disease prevalent in the tropics. This study was carried out to estimate the seroprevalence of lymphatic filariasis in and around Puducherry using indirect haemagglutination test. Of the 5056 clinically suspected cases, 2214 (43.78%) were found to be positive, of whom majority were males (57.8%) belonging to the age group of 21–40 years, indicating that lymphatic filariasis mainly affects the adolescents and adults mainly. This high seroprevalence is a matter of immediate concern and necessary control programme is urgently required to check the transmission of filariasis.

Keywords Lymphatic filariasis · Indirect haemagglutination assay · Seroprevalence

Introduction

Lymphatic filariasis (LF), a mosquito vector-borne disease is a major public health problem in many parts of the tropics. The term “lymphatic filariasis” denotes infection by *Wuchereria bancrofti* and *Brugia malayi*. It is a major health problem affecting about 250 million persons in tropical countries. The condition is endemic in 80 countries, and more than two billion people worldwide are estimated to be at risk. Approximately 128 million people in tropical and subtropical areas of the world are infected (Parija 2005). The largest number of cases of filariasis

occurs in India, where over 300 million people live in endemic zones. It is estimated that at least 6 million attacks of acute filarial disease occur every year in India and that over 15 million persons have chronic filarial disease (Agrawal and Sashindran 2006).

Infection with filarial worm can cause wide variety of clinical manifestations, ranging from those without apparent clinical diseases to those with lymphedema and/or severe disfigurement of the limbs and genitalia. The majority of filarial infected individuals in endemic areas have few clinical manifestation of the filarial infection despite large numbers of circulating microfilariae in the peripheral blood (Ottesen 1992). To study the prevalence of lymphatic filariasis from a given region the method used by National Institute of Communicable disease is to examine 5–7% of population for routine survey and at least 20% for evaluation studies, and various parasitological indicators like microfilaria rate, filaria endemecity rate and microfilaria density are evaluated. The other methods available are estimation of seroprevalence by indirect hemagglutination assay (IHA), indirect fluorescent antibody test (IFAT) and enzyme linked immunosorbent assay (ELISA). Both microscopy and seroprevalence test have their inherent advantages and disadvantages (Garcia and Bruckner 2007). Since lymphatic filariasis is a major public health problem in southern India, we have studied seroprevalence of lymphatic filariasis in and around Puducherry using in house standardized indirect hemagglutination assay.

Materials and methods

Serum samples were collected from December 2008–February 2009 and studied by IHA test, from patients attending JIPMER hospital, Puducherry, India, The reasons

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for clinical suspension of filariasis ranged from mild cough and urticaria to those with severe lymph edema and/or severe disfigurement of the limbs and genitalia. The serum was stored at -20°C till the performance of test.

Preparation of filarial antigens and optimization of sensitizing dose calculation

Adult female *Setaria digitata* worms were collected from peritoneal cavity of cattles from slaughter house. The isolated worms were grounded in sterile mortar and pestle followed by sonication. The suspension was then centrifuged at 12,000 rpm for 30 min; supernatant was separated and used as antigen for IHA test. optimum sensitizing dose (OSD) was determined for each dilution of stabilized cells by chequer board titration against the known positive control and negative control as described earlier (Parija 2005; Parija and Sahu 2005).

Stablization and sensitization of chick red blood cells

Chick RBC were collected in alsever's solution and stabilized by double aldehyde stabilization method (Parija et al. 1986). To sensitize these stabilized cells, 0.1 ml of stabilized chick RBC were mixed with 0.9 ml of OSD of antigen. The mixture was kept in water bath for 5 min at 50°C with intermittent shaking. Followed by overnight incubation at 4°C . Next day 1% suspension was made with 0.1% bovine serum albumin and used in test.

Performance of IHA filariasis test

A 25 μl volume of the diluent (PBS pH 7.2 with 0.1% bovine serum albumin) was initially dispensed in each well of U bottomed microtitre tray. Then 25 μl volume of the serum was added to the first well of the appropriate rows. The sera were then serially diluted up to the eleventh well leaving the last well as a serum free control. 25 μl volume of the 1% cells sensitized with the OSD antigen was added to each well. The plate was gently agitated for 2 min and incubated at room temperature for 1/2 h and the reading was taken. Formation of red matt at bottom of microtitre plate with light red colour supernatant was taken as positive result, whereas dot formation at base of microtitre plate with clear supernatant was taken as negative result. A positive cut off titer was taken as taken as 64 (Parija 2005).

Validation of IHA filariasis test

To rule out false positive reactions, IHA test was performed on ELISA confirmed positive serum samples of patients with malaria, toxoplasmosis, amoebiasis, neurocysticercosis, hydatid disease, typhoid fever and brucellosis. The IHA

was negative in all above serum samples. For checking false positive reactions, 10 serum samples from clinically diagnosed cases of lymphatic filariasis were taken and seropositivity was confirmed by recombinant antigen (WbSXP-1) based Immunospot test. (Span diagnostics, India). IHA test was positive above cut off titer in all 10 serum samples.

Results

A total 5056 serum samples were tested for filarial antibody by IHA test. Of them 2214 (43.78%) samples were positive. In positive samples 1277 (57.68%) samples were from males and 937 (42.32%) from female. The age groups were also studied and results showed that 21–40 years old patients were most common followed by 41–60 years old. (Table 1) The common symptoms due to which samples were collected from patients, included urticaria, filarial limbs, epididymorchitis/scrotal swelling/hydrocele, cough, pedal edema (pitting/non pitting), tropical pulmonary eosinophilia, fever, bronchial asthma and others (Table 2).

Discussion

Lymphatic filariasis is a global problem. It is a major social and economic scourage in the tropics and subtropics of Africa, Asia, Western pacific and parts of the Americas, affecting over 120 million people in 80 countries. More than 1.1 billion people live in areas where there is a risk of infection (WHO 1998). It is also a major public health problem in India. The disease is endemic all over India and surveys carried out during the past two decades indicate that area previously known to be free from filariasis are showing evidence of low degrees of transmission. Heavily infected areas are found in Uttar pradesh, Bihar, Jharkand, Andra pradesh, Orissa, Tamil Nadu, Kerala, and Gujarat. (WHO 2006).

Singh et al. (1988) did an IHA test on 173 serum samples obtained from patients and persons exposed to

Table 1 Seropositivity of IHA filariasis in different age group at Puducherry

Age	Number of positives By IHA	Percentage (%)
0–20	175	7.9
21–40	1204	54.4
41–60	682	30.80
Above 60	153	6.9
Total positive	2214	43.7
Total patients tested by IHA	5056	

Table 2 Clinical symptoms of filaria affected patients in Puducherry

Symptoms of patients	Males	Females	Total (%)
Urticaria	164	161	325 (14.67)
Filaria/filarial limb	331	261	592 (26.73)
Epididymorchitis/scrotol swelling/hydrocele	185	0	189 (8.35)
Cough	25	15	40 (1.80)
Pedal edema/pitting/nonpitting	85	39	124 (5.60)
Tropical pulmonary eosinophilia	145	111	256 (11.56)
Fever	16	19	35 (1.58)
Bronchial asthma	37	49	86 (3.88)
Lymphadenopathy	20	15	35 (1.58)
Other symptoms (include chyluria, air-borne dermatitis, psoriasis, bulbous disease, erythroderma etc.)	269	267	536 (24.20)
Total number of positive samples by IHA test	1277	937	2214
Total samples tested by IHA	3022	2034	5056

W. bancrofti and *B. malayi* in endemic areas of Peninsular Malaysia. They reported that, positive IHA test rates in sera from microfilaria-negative persons in endemic areas, microfilaremic cases, and patients with clinical filariasis were 13, 75, and 80%, respectively (Singh et al. 1988). In a similar study from West-Indies, Rawlins et al. (1994) collected venous blood from 292 patients attending a Filaria Clinic in Georgetown, Guyana, and assayed by ELISA for IgG and IgM antibodies and by indirect hemagglutination Assay against filarial parasites. They reported that of the 41 blood samples microscopically positive for *Wuchereria bancrofti* microfilariae, seropositivity was seen in 87.8% by ELISA IgG, 65.9% by ELISA IgM and 73.2% by IHA (Rawlins et al. 1994).

There are various studies from India regarding prevalence of lymphatic filariasis. A survey was conducted in July 1998 in Pathankot town of Punjab and a total of 2136 blood smears were collected from migratory and local inhabitants. Microfilaria (Mf) rate and mean Mf density was 1.19 and 15.05, respectively. Mf rate was highest in 20–49 years age-group, whereas Mf density was high in younger age group (Singh et al. 2000). Das et al. performed study of lymphatic filariasis from Patna district in Bihar, a area known to be endemic for lymphatic filariasis. Of the 1872 persons examined, 8.4% were found asymptomatic but microfilaraemic. Morbidity pattern due to filarial infection showed an increase with advancement of age and significantly high in males as compared to female. Acute and chronic filarial disease was observed as 0.5 and 9% respectively. Microfilaria was found in 10% of acute and 11.2% of chronic filarial cases (Das et al. 2006).

In our study IHA filariasis test was done on 5056 serum samples. Of which 43.78% samples were positive. In another studies from Madhya Pradesh, India similar results were reported (Das et al. 2005). The infection was more common in male as compared to females. Such high prevalence in

males has been reported in other studies (Singh et al. 1988; Das et al. 2005) probably because of habit of males of sleeping outside in rural areas and partly due to field works.

The age groups were also studied and it was seen that seroprevalence increased with age with peak between 21–40 year old patients, followed by 41–60 years old patients. Similar age distribution is also documented in other studies (Parija and Sahu 2005; Das et al. 2005) and is probably because this age group is the main work force involved in working outside house and getting exposed to mosquito bites.

To conclude, about 42% per seroprevalence at Pondicherry is a matter of immediate and prompt and complete implementation of filarial control programme in south India is need of the hour to check the transmission of lymphatic filariasis.

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