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Potentiality of Enzymelinked Immunosorbent Assay (ELISA) using Adult *Brugia malayi* Antigen in the Diagnosis of Lymphatic Filariasis

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Abstract

An enzymelinked immunosorbent assay (ELISA) developed using the soluble antigen of adult *Brugia malayi* and alkaline phosphatase conjugate of anti-human IgG was evaluated for its diagnostic potentials in an area endemic for Wuchereriasis. The procedure gave positive response in about 95% cases (n=234) of clinical filariasis, and 80% cases (n=85) of asymptomatic microfilaraemic individuals. None of the control subjects (20) from nonendemic area showed positive response by this procedure. Although cross reaction of the test was not observed in patients with hookworm, *Giardia, Entamoeba histolytica* and tapeworm infections, about 16% subjects from Kashmir who were heavily infested with *Ascaris* gave weak but positive response.

Key words: Lymphatic filariasis, Wuchereriasis, Immunodiagnosis, *Brugia malayi* antigen, Enzymelinked immunosorbent assay

Introduction

The diagnostic test for lymphatic filariasis, based on the examination of night blood smear for the presence of microfilariae has limitations, since in a large number of clinical cases microfilariae are not detectable in peripheral night blood. Most of the immunological tests employed so far for the diagnosis suffer from the drawback that either they are insensitive or give positive response in apparently healthy nonfilarial subjects of endemic area due to sensitization resulting from constant exposure to infection or cross-reaction with other parasites. Enzymelinked immunosorbent assay (ELISA) has been proven as the sensitive test and used for the diagnosis of several parasitic diseases (Voller *et al.*, 1976; Saunders and Clinard, 1976). ELISA using antigens of adult *L. carinii,* the filarial parasite of rodent and dot ELISA using antigen of adult *B. malayi,* the filarial parasite of man, have been reported from this laboratory (Tandon *et al.*, 1981; Saxena *et al.*, 1981; Tandon *et al.*, 1988).

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The present paper describes the results of a study carried out on the evaluation of microtitre plate ELISA with *B. malayi* adult antigen on a number of clinical cases and asymptomatic carriers of filariasis.

Materials and Methods

Study groups — Evaluation of ELISA was carried out in four groups of subjects, namely (1) clinical cases of wuchereriasis represented by lymphangitis and lymphadenitis of lower and upper limbs, hydrocoele, elephantiasis and chyluria accompanied by a history of chill and fever (2) asymptomatic carriers of microfilariae (3) nonfilarial healthy subjects of endemic area, and (4) nonfilarial healthy subjects from nonendemic area (West Germany).

Microfilaraemia was established by examination of night blood smear (20 cmm) taken at mid night. In control subjects of endemic area absence of microfilariae (mf) in nocturnal blood (2 ml) was confirmed by concentration technique using Millipore filter membrane of 0.45 μ pore size. The patients and controls were in the age group of 16–50 years.

Collection of blood: About 1 ml blood was collected by vein puncture. The serum separated and stored frozen until used.

Preparation of antigen and method of ELISA

Brugia malayi adult worms, collected from infected *Mastomys natalensis,* were cut into small pieces and homogenized with ten times its weight of chilled 0.15M sodium chloride in a Potter Elvehjem homogenizer fitted with teflon pestle: Homogenate was frozen, thawed thrice and somicated at 20 KC/Sec (Sonicator™ HEAT SYSTEMS — ULTRASONICS, INC. Model W-220F). It was finally spun in an ultracentrifuge (Beckman Ultracentrifuge Model L) at 105,000 × g for an hr and the supernatant was used as soluble antigen.

Protein concentration was estimated by the method of Lowry *et al* (1951) and the antigen stored in aliquots at −70°C.

ELISA was carried out essentially by the method of Engvall and Perlmann (1972) with slight modifications as described by Tandon *et al* (1981). Disposable polystyrene micro ELISA strips (VIRION MT-Strips) were used for coating the antigen; 200 µl of antigen solution (5 ng protein/well) in 0.05 M sodium carbonate buffer (pH 9.6) containing NaN_3 was added to each well. The strips were kept in humid chamber at room temperature for an hr and then left overnight at 4°C. Next day wells were washed with 0.01 M phosphate buffered saline (PBS pH 7.4) containing 0.05% Tween 20 (Sigma Chemical Co. St. Louis. U.S.A.). Two hundred microlitre of serum (1:500 diluted in PBS Tween) was dispensed into duplicate wells and incubated for 2 hr in humid chamber at room temperature, the wells were again washed and 200 µl of antihuman IgG Alkaline phosphatase conjugate (Sigma Chemical Co. St. Louis. U.S.A.; 1:1000 diluted in PBS Tween) was added to each well. After 2 hr incubation at room temperature, the wells were washed and 200 µl of substrate p nitrophenyl-phosphate (Sigma Chemical Co. St. Louis. U.S.A.) made in 0.1 M sodium carbonate buffer (pH 9.8) was added. The absorbancy of the yellow coloured p-nitrophenol liberated as a result of the enzyme hydrolysis was measured at 405 nm using ELISA Reader (VIRION auto Reader A, INSTITUT VIRION Würzburg).

Results

ELISA values in filarial and nonfilarial groups and ELISA positivity

Table 1 shows the mean ± SD ELISA values in terms of extinction of p-nitrophenol liberated by the enzyme reaction at 405 nm and ELISA positivity among filarial and nonfilarial groups of subjects. The European control subjects from West Germany had negligible ELISA values, while the nonfilarial subjects of endemic area had

Table 1. ELISA values with *B.malayi* antigen

Study group	ELISA* positive cases		ELISA value E/405	
	No.	%	Range	Mean ± SD
Control subjects				
Endemic (n = 50)	4	8	0.068–0.240	0.118 ± 0.049
Nonendemic				
European (n = 20)	Nil	–	0.040–0.080	0.060 ± 0.012
Filariasis cases				
Symptomatic (n = 234)	222	95.1	0.184–1.176	0.586 ± 0.297[a]
Asymptomatic (n = 85)	68	80	0.053–0.687	0.319 ± 0.124[a]

[a] = $P < 0.001$ (values compared to the values of endemic controls)
* = ELISA value > mean + 2 SD of endemic controls

a low mean ± SD ELISA value of 0.118 ± 0.049 with a range of 0.068–0.240. The mean ± SD ELISA values of the clinical cases and asymptomatic microfilaraemic individuals was significantly higher being 0.586 ± 0.297 and 0.319 ± 0.124 respectively. The differences between the ELISA values of normal endemic and filarial subjects were statistically significant (P values < .001 for clinical and asymptomatic cases).

Considering ELISA values greater than mean + 2SD of healthy nonfilarial subjects of endemic area as positive for filariasis, 95.1% subjects among the clinical cases and 80% among the asymptomatic carriers were positive for the disease. Among the endemic controls 8% of the subjects gave positive ELISA response while among the non-endemic West German controls none showed positive ELISA response.

ELISA positivity in clinical cases with different manifestations

Data on the mean ± SD ELISA values and ELISA positivity among the clinical cases with different manifestations have been presented in Table 2. 87 out of 90 (96.6%) cases of acute filariasis in the form of lymphangitis/lymphadenitis of lower and upper limbs and 80 out of 84 (95.2%) cases of filarial hydrocoele gave positive response to ELISA. Among 36 cases having both clinical manifestations and microfilaraemia 34 (94.1%) and among 15 cases of full fledged elephantiasis 12 (80%) showed positive ELISA response. The 3 cases of elephantiasis which gave negative response to ELISA had history of filariasis of over 20 years each. All the 6 cases of elephantiasis with hydrocoele and 3 cases of chyluria gave positive ELISA response.

Cross reaction with other parasite infestations

Sera from subjects harbouring certain helminth and parasitological infestations were examined for their possible cross reactivity with filariasis in ELISA. As depicted in Table 3 none of the sera from subjects harbouring *Ascaris* (10), *Ancylostoma* (10), *Taenia* (8), *Giardia* (10) or *Entamoeba histolytica* (10) gave a positive ELISA response. However out of 30 sera from subjects heavily infested with *Ascaris,* collected from Kashmir (Srinagar) 5 gave mildly positive ELISA response.

Discussion

The results presented above show that ELISA

Table 2. ELISA positivity in different groups of clinical cases

	Total	ELISA positive cases No.	%	ELISA value E/450 Range	Mean ± SD
1. Lymphangitis/ Lymphadenitis of upper or lower limb	90	87	96.6	0.246–1.176	0.547 ± 0.293
2. Hydrocoele	84	80 (3 operated)	95.2	0.217–1.098	0.653 ± 0.280
3. Clinical manifestations with microfilaria	36	34	94.1	0.184–0.936	0.544 ± 0.213
4. Elephantiasis	15	12	80	0.233–1.137	0.568 ± 0.264
5. Elephantiasis & Hydrocoele	6	6	100	0.337–0.704	0.568 ± 0.195
6. Chyluria	3	3	100	0.337–0.529	0.508 ± 0.104

Table 3. ELISA values of Kashmir controls, and subjects harbouring *Ascaris, Taenia, Giardia, Ancylostoma* and *Entamoeba histolytica*

Infestations	Numbers		ELISA value E/405	
	Total	ELISA positive	Mean ± SD	Range
1. Ascariasis	10	0	0.136 ± 0.034	0.096–0.198
2. Taeniasis	8	0	0.131 ± 0.031	0.096–0.195
3. Giardiasis	10	0	0.099 ± 0.032	0.090–0.170
4. Ancylostomiasis	10	0	0.096 ± 0.006	0.086–0.106
5. Amoebiasis	10	0	0.102 ± 0.03	0.068–0.170
6. Kashmir control	30	5	0.159 ± 0.099	0.008–0.456

standardised with adult *B. malayi* soluble antigens was able to confirm filariasis in about 95% of the cases with clinical manifestations. Manifestationwise the positivity was highest (96.6%) among acute cases of lymphangitis and lymphadenitis of lower and upper limbs. Among the hydrocoele cases the positivity was slightly lower but if one excludes the three operated cases the ELISA positivity would be 98.7%. The procedure could establish the disease in all cases of chyluria and having elephantiasis with hydrocoele. However, the number of subjects in the latter two groups was too small to draw any definite conclusion at this stage. Among the clinical cases the positivity was lowest in the elephantiasis group where only 80% cases gave positive ELISA response. It may however, be pointed out that 3 out of 15 elephantiasis cases which gave negative response to the procedure had prolong history of over 20 years and it is likely that inspite of swelling which is irreversible in chronic cases the parasite had been dead for long time and there was no antigen to induce antibody. Only 80% of the asymptomatic carriers gave positive response to ELISA. Failure to obtain positive ELISA response in the remaining 20% of subjects inspite of the presence of microfilariae was presumably due to the immunosuppression (Grove and Davis, 1978; Saxena *et al.*, 1981; Ottesen, E.A., 1984; Nutman *et al.*, 1987) existing in these individuals.

None of the West German controls showed positive ELISA response, but 8% of the healthy nonfilarial individuals from the endemic area showed ELISA response of greater than mean + 2 SD of normal endemics. This could be due to sensitization of the individuals as a result of constant exposure to the bite of infected mosquitoes or probably due to some cross reacting infection. Cross reaction of ELISA was, however, not found with ancylostomiasis, taeniasis, giardiasis, amoebiasis or ascariasis in subjects from endemic areas. Some of the heavily infested cases of ascariasis drawn from Kashmir showed false positive response by cross reaction.

Among various possible factors that may influence the specificity and sensitivity of an immunological test, the source of antigen, its amount, and the dilution of the test serum used play important roles. Various immunological tests developed so far also give positive response in a significant percentage of asymptomatic, amicrofilaraemic healthy subjects of endemic areas (Ambroise Thomas, 1974; Grove and Davis, 1978; Dissanayake and Ismail, 1981; Kaliraj *et al.*, 1981; Kharat *et al.*, 1982). The ELISA developed with *B. malayi* adult antigen under the assay conditions employed gave low ELISA values with the sera of normal subjects from the endemic area than those from filariasis patients. The difference between the ELISA values of the two groups was statistically highly

significant. These observations are in agreement with those of Grove and Davis (1978) and Kaushal *et al.* (1984), who have shown negligible or very low reactivity of adult *B. malayi* antigen with the sera from endemic normals and subjects harbouring Ascaris.

ELISA developed so far, for identification of filarial antibodies have different sensitivities. ELISA using antigens from heterologous filarial parasites, namely *Litomosoides carinii* (Tandon *et al.*, 1983; Saxena *et al.*, 1981) and *Dirofilaria immitis* (Sengupta *et al.*, 1985) have been reported to elicit positive response in 90% and 88.8% of the clinical cases respectively. ELISA with *W. bancrofti* microfilariae soluble (Kaliraj *et al.*, 1982) or culture filtrate (Kharat I. *et al.*, 1982; Malhotra *et al.*, 1982) antigens have shown varying responses in clinical cases of filariasis and endemic normal subjects. Stick ELISA (Parkhe *et al.*, 1986) using microfilariae culture antigens have shown positive response in all the 12 clinical cases, about 91% microfilaria positive cases and 17% normal subjects of endemic area. Since the filarial subjects in the present study were grouped as symptomatic (both mf positive and mf negative) and asymptomatic (mf positive) a close comparison of the results of the study with those carried out with microfilarial antigen was not possible.

ELISA with *B. malayi* adult stage antigens thus holds promise in diagnosis of filariasis. Studies on further purification of diagnostic antigens are underway.

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