J. Biosci., Vol. 4, Number 4, December 1982, pp. 507-512.

Detection of filarial infection using *Wuchereria bancrofti* microfilariae culture antigen and filter paper blood samples in enzyme linked immunosorbent assay

ASHOK MALHOTRA, M. V. R. REDDY, J. N. NAIDU, S. N. GHIRNIKAR* and B. C. HARINATH

Department of Biochemistry, M. G. Institute of Medical Sciences, Sevagram, Wardha 442 102

* Research cum Training Centre, Wardha 442 001

MS received 11 May 1982; revised 16 August 1982

Abstract. Blood collected on filter paper by finger-prick gave results comparable to intravenous serum samples when analysed by enzyme-linked immunosorbent assay (ELISA). All the 100 microfilaraemia, 5 out of 100 endemic normals and none of the 10 nonendemic normal filter paper blood samples showed the presence of filarial antibody when tested by this method,using culture antigen and anti-immunoglubulins, class G, M and A — penicillinase conjugate. When the same samples were screened for the presence of IgM antibody, 91 out of 100 microfilaraemia, 13 out of 100 endemic normal and none of the 10 nonendemic normal samples showed a positive reaction. Enzyme linked immunosorbent assay, using culture antigen and filter paper blood samples, appears to work in large field studies for detection of filarial infection.

Keywords. Filter paper blood samples; enzyme - linked immunosorbent assay (ELISA); culture antigen.

Introduction

Blood samples collected and dried on filter paper have been shown to be useful in seroepidemiological studies for detection of parasitic diseases such as malaria (Lobel *et al.,* 1976), onchocerciasis (Ikeda *et al.,* 1978), leishmaniasis (Al-Aloust *et al.,* (1980), amoebiasis (Mathews *et al.,* 1980) and trypanosomiasis (Roffi *et al.,* 1980). These filter paper blood samples are even more relevant and will be advantageous in nocturnally periodic bancroftian filariasis, where it is difficult to collect night blood smears and in field studies. Kharat *et al.* (1982) have shown that *Wuchereria bancrofti* microfilariae culture antigen is highly sensitive in detecting filarial antibody by enzyme linked immunosorbent assay (ELISA). This study reports the use of filter paper blood samples in immunodiagnosis of human filariasis by ELISA using *W. bancrofti* microfilariae culture antigen.

Abbreviations used: ELISA, Enzyme-linked immunosorbent assay, PBS, phosphate buffer saline; BSA, bovine serum albumin; PBS/T, PBS containing 0.05% Tween 20.

Materials and methods

Sera

Venous blood was collected from filarial patients (microfilariae—positive) and normal individuals (microfilariae—negative) residing at Sevagram and its surrounding villages which is endemic for nocturnally periodic bancroftian filariasis. Serum was separated and stored at—10°C after addition of sodium azide (0.1%) as preservative. Nonendemic normal blood samples were collected from students coming to this institute from nonendemic regions such as Chandigarh, Kashmir etc. Microfilaraemia was confirmed by wet blood smear examination.

Filter paper blood specimens

Twenty µl of blood was collected by finger prick with a pipette and transferred immediately to Whatman No. 3 filter paper and was allowed to dry at room temperature. It was observed that 20 µl of blood covers a circle of diameter of about 0.9 cm. Hence the blood was collected directly on to filter paper by finger prick in such a way that it covers a circle of diameter of more than 0.9 cm. Blood on filter paper was dried at room temperature. From this a circle of 0.9cm diameter was cut for elution. Filter paper with dried blood sample was cut into small pieces and eluted into 0.75 ml of 0.5 M phosphate buffer saline (PBS), pH 7.2 into a tube by shaking at 37°C for 2 h. After centrifugation at 600 *g* in a clinical centrifuge for 10 min., the supernatant was separated and stored at—10°C. Sodium azide (0.1%) was used as preservative. Undiluted filter paper eluate was assigned an equivalent serum dilution of 1:75, assuming serum content of blood as 50%.

Enzyme linked immunosorbent assay

Penicillinase (specific activity 340 I.U./mg protein) and penicillin V were obtained from Hindustan Antibiotics, Pune . Anti-human immunoglobulin IgG+IgM+ IgA as well as individual anti-human IgM (Immunodiagnostics, New Delhi), bovine serum albumin (Sigma Chemical Co., St. Louis, Missouri, USA) and polyvinyl microtitre plates (Dynatech Laboratories) were used in this study. *W. bancrofti* microfilariae culture antigen was kindly supplied by Miss Kharat of this department. The procedure for preparation of culture antigen (Kharat *et al.,* 1982) in brief, consists of separation of *W. bancrofti* microfilariae from microfilaraemia blood and maintainance for 15 days in Medium 199 (Paul, 1975) supplemented with organic acids and sugars of Grace's medium (Paul, 1975). The culture fluid collected every 24 h, was centrifuged at 13,000 g for 15 min and the supernatant was stored at–20°C and used when required. The protein in the culture fluid was estimated by Lowry's method (Lowry *et al.,* 1951).

Substrate in ELISA consisted of soluble starch (150 mg) in 27.5 ml of sodium phosphate buffer 0.2 M, pH 7.0 containing 10.64 mg of penicillin V and 65 µl of 0.08 M iodine in 3.2 M potassium iodide solution. The substrate was prepared fresh before use.

Conjugation of antihuman Ig and penicillinase was achieved by the method of Avrameas (1969) using glutaraldehyde. The optimum working dilutions of culture

antigen and penicillinase anti human Ig conjugates were determined by chequer board. titration.

ELISA was carried out as described by Kharat *et al.* (1982) with some modifications. To the wells in the microtitre plate were added 100 µl of optimally diluted culture antigen (3.5 ng of protein per ml of carbonate buffer 0.06 M, pH 9.6) incubated at 37°C for 3 h and then drained. The plate was further incubated with 200 µl of 3% bovine serum albumin (BSA) in the same carbonate buffer at 37° C for 2 h. It was then washed 8 times with PBS 0.01M, pH 7.2 containing 0.05% Tween 20 (PBS/T). Hundred microlitres of test serum (1:300) diluted in PBS/T or same volume of equivalent blood sample dilution (in PBS/T) were added, incubated at 37°C for 3 h or overnight at 4°C, followed by washing. Then 200 µl of 3% BSA in PBS/T was added to each well, incubated at 37°C for 1 h. After washing the plate again, penicillinase antihuman IgG+IgM+IgA (1:50) conjugate of 100 µl volume was incubated in each well at 37°C for 3 h. After a thorough washing, the plate was incubated with 100 µl of substrate at 37°C for 1 h. The reaction was terminated by the addition of 25 µl of 5N HCl and the results were evaluated visually. The disappearance of blue colour of substrate was taken as positive reaction. The assay were done in triplicate.

Additional studies were carried out with penicillinase labelled anti human IgM (1:10). Kharat *et al.* (1982) observed that 3 out of 20 nonendemic normal sera gave a positive reaction at serum dilution of 1:160 when screened for antibody by ELISA using culture antigen and penicillinase conjugated with anti IgG+IgM+ IgA. Hence we considered 1:300 titre as positive reaction for filarial antibody using this test system for screening of filarial sera.

Results

Twelve human blood samples belonging to microfilaraemia and control groups collected by different methods [intravenous serum (a), eluate from filter paper blood spot containing 20 µl of blood (b), and eluate from filter paper blood spot of 0.9 cm diameter (c)] were screened at the serum or equivalent blood sample dilution of 1:300 for the presence of filarial antibody by ELISA using culture antigen and penicillinase conjugated with IgG+IgM+IgA. Table 1 shows that the 3 types of samples i.e. a, b and c gave identical results when examined at the cut off dilution of 1:300. While all of the 8 microfilaraemia samples showed the presence of filarial antibody none of the 4 nonendemic normal samples were positive.

A total number of 210 human blood samples collected on filter paper belonging to different groups were screened at the equivalent serum dilution of 1:300 by ELISA using culture antigen and penicillinase conjugated with anti IgG+IgM+ IgA and the results are summarized in table 2. All the 100 microfilaraemia and 5 out of 100 endemic normal samples tested showed the presence of filarial antibody but none of the 10 nonendemic normal samples were positive. To detect the presence of class specific IgM antibody, the same samples were screened at the equivalent serum dilution of 1:300 by ELISA using culture antigen and penicillinase conjugated with anti IgM. Nintyone out of 100 microfilaraemia, 13 out of 100 endemic normal and none of the 10 nonendemic samples showed the presence of

Table 1. Comparison of sera and filter paper blood samples for detection of antioodies in ELISA using culture antigen.

Sera/blood	No. screened	No. showing positive reaction*		
		Sera	Filter paper eluate from	
			Known volume of blood (20 µl)	Known area covered by blood (circle of 0.9 cm diameter)
Non endemic normal (mf −ve)	4	0	0	0
Microfilaraemia (mf +ve)	8	8	8	8

* Sample showing positive reaction for filarial antibody at serum or equivalent serum dilution of blood sample at 1: 300

Table 2. Analysis of microfilaraemia blood dried on filter paper by ELISA using culture antigen.

Blood	No. screened	No. showing positive reaction* with	
		anti IgG+IgM+IgA conjugate	anti IgM conjugate
Non endemic normal (mf −ve)	10	0	0
Endemic normal (mf −ve)	100	5	13
Microfilaraemia (mf +ve)	100	100	91

* Sample showing positive reaction at equivalent serum dilution of the blood sample at 1:300,

IgM antibody. The 5 endemic normals which showed antibody with mixed conjugate have also shown the presence of IgM antibody along with 8 more endemic normal samples.

Discussion

During sero epidemiological mass investigations of trypanosomiasis, Roffi *et al.* (1980) have found the combination of filter paper blood samples and ELISA a

valuable diagnostic tool. In filariasis, as far as we are aware, this is the first attempt to establish the utility of blood samples collected on Whatman No 3 filter paper in detection of antibody, using culture antigen in ELISA. Thus the major problem of collecting night blood samples in the field for diagnosis of filariasis can be avoided.

The diagnosis of filarial infection based on antibody detection has been frustrating because of difficulty in identifying an active infection from an exposed individual who is not having infection. In an endemic region even when soluble *W. bancrofti* microfilariae antigen was used, 55% of endemic normals showed the presence of filarial antibody (Kaliraj *et al.,* 1981). However, when *W. bancrofti* microfilariae culture antigen was used, all of the 100 microfilaraemia and only 5 of the 100 endemic normal filter paper samples showed filarial antibody with mixed conjugate. This is encouraging and agrees with observations made by Kharat *et al.* (1982) using much smaller number of serum samples.

We have observed the presence of IgM antibody in 91 out of 100 microfilaraemia cases (table 2). Lunde *et al.* (1980) have shown the presence of specific anti egg antigen IgM in all the 13 patients of early schistosomiasis thereby indicating that IgM is associated with active infection. Absence of specific anticulture antigen IgM in 9 microfilaraemia cases may be due to the transition of these cases into chronic stage where IgM antibody is mostly absent. However they did not show any early clinical manifestations when examined. Five out of the 13 endemic normals who showed the presence of IgM antibody (table 2) when further examined for microfilariae by concentration test were found to be negative. Three of these endemic normals were further examined by diethylcarbamazine (2 mg/kg body wt) provocative test and were found to be negative, These persons will be followed by periodic examination, to see whether they will become microfilaraemic in due course of time.

Denham *et al.* (1971) have shown that 30% of the microfilariae were lost at various stages of preparing and staining blood films. Southgate (1973) estimated this loss to be 50% under field conditions by experienced filariasis technicians. In the present study, by doing the wet blood smear examination in the field itself, the possibility of missing microfilaraemia cases was minimized. We still got 5 more cases out of 100 endemic normals positive by ELISA with mixed conjugate. Collection of blood samples directly on to the filter paper even in day time is an added advantage and convenient as compared to wet blood smear examination in the field at night.

Combination of filter paper blood samples and ELISA using culture antigen and mixed conjugate appears to work satisfactorily in detection of filarial infection (only 5% false positive reaction based on wet blood smear examination) in large scale field studies for undertaking drug treatment in areas endemic for bancroftian filariasis.

Acknowledgements

One of the authors (A.M.) is grateful to Indian Council of Medical Research for awarding Senior Research Fellowship. We thank Dr. Sushila Nayar, and Dr M. L.

Sharma, for their keen interest and encouragement. Thanks are also due to Mr. Demapure for help in collection of filarial blood samples and to Messrs Ankar, Zumde and Vaidya for technical assistance. This study was supported in part by a grant from UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases.

References

Al-Aloust, T. I., Latif, B. M. A. and Al-Shenawi, F. A. (1980) *Ann. Trop. Med. Parasitol.,* **74,** 503.

Avrameas, S. (1969) *Immunochemistry,* **6,** 43.

Denham, D. A., Dennis, D. T., Ponnudurai, T., Nelson, G. S., and Guy, F.(1971) *Trans.Roy.Soc.Trop. Med. Hyg.,* **65,** 521.

Ikeda, T., Tada, I., and Aoki, Y. (1978) *J. Parasitol.,* **64,** 786.

Kaliraj, P., Ghirnikar, S. N., and Harinath, B. C. (1981) *Am. J. Trop. Med. Hyg.,* **30,** 982.

Kharat, I., Ghirnikar, S. N. and Harinath, B. C. (1982) *Indian J. Exp. Biol.,* **20,** 378.

Lobel, H. O., Nzera, A. J., Ch'en, W. I., Munroe, P. and Mathews, H. (1976) *J. Trop. Med.,* **79,** 275.

Lowry, O. H., Rosebrough, H. J., Farr, A. L. and Randall, R. J., (1951) *J. Biol. Chem.,* **193,** 265.

Lunde, M. N. and Ottensen, E. A. (1980) *Am. J. Trop. Med. Hyg.,* **29,** 82.

Mathews, H. M., Spencer, H. C. and Healy, G. R. (1980) *Trans. Roy. Soc. Trop. Med. Hyg.,* **74,** 404.

Paul, J. (1975) in *Cell and tissue culture,* (Edinburg, London, New York: Churchill Livingstone) p. 439, 448.

Roffi, J., Carrie, J., Garre, M. T., and Dedet, J. P. (1980) *Bulletin de la socie'te'de Pathologie Exotique,* **73,** 67.

Southgate, B. A. (1973) *S. E. Asian J. Trop. Med. Pub. Hlth.,* **4,** 172.