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Enzyme-linked immunosorbent assay for the diagnosis of *Wuchereria bancrofti* infection using urine samples and its application in Bangladesh

Mohammad Sohel Samad [a], Makoto Itoh [a,*], Kazuhiko Moji [b], Moazzem Hossain [c], Dinesh Mondal [d], Mohammad Shafiul Alam [d], Eisaku Kimura [a]

[a] Department of Infection and Immunology, Aichi Medical University School of Medicine, 1-1 Yazakokarimata, Nagakute, Aichi 480-1195, Japan
[b] Research Institute for Humanity and Nature (RIHN: Chikyu-ken), 457-4 Motoyama, Kamigamo, Kita-ku, Kyoto 603-8047, Japan
[c] Institute of Allergy & Clinical Immunology of Bangladesh (IACIB), Green Super Market, Green Road, Dhaka 1205, Bangladesh
[d] International Center for Diarrheal Disease and Research, Bangladesh (icddr,b) 68, Shahid Tajuddin Ahmed Sharani, Mohakhali, Dhaka 1212, Bangladesh

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ABSTRACT

In Sri Lanka, urine ELISA showed high sensitivity and specificity in detecting filaria-specific IgG4. It also produced much higher positive rates than antigen tests in prevalence studies with young children. In this study, we have confirmed the usefulness of urine ELISA in the field of Bangladesh. The ELISA detected 89 of 105 (85%) ICT antigen test positive subjects in endemic areas. With both ICT and microfilaria positives, the sensitivity was 97% (30/31). All of 104 ICT negative people in a non-endemic area were ELISA negative (100% specificity). In a prevalence study with 319 young children (5–10 years) from a low endemic area after five rounds of MDA, seven (2.2%) were detected by the present urine test, but only one (0.3%) by ICT ($P = 0.075$). The satisfactorily high sensitivity, 100% specificity and effective case detection among young ages along with scope for analyzing the titers will indicate urine ELISA to be an effective tool in the post-MDA surveys to confirm elimination or to detect resurgence in Bangladesh.

1. Introduction

Lymphatic filariasis is a significant public health concern in endemic countries especially in Asia and Africa. Causing long-term disability in tens of millions of people, it has been a major obstacle to socioeconomic development [1–3]. Globally, more than 120 million people in 81 countries are reported infected with 1.3 billion people at risk of infection [4–6].

In 1997, WHO made a resolution to eliminate the disease from the world, and the Global Programme to Eliminate Lymphatic Filariasis (GPELF) has been implemented since 2000 with the target of elimination by the year 2020. The basic strategy is to treat all eligible people in endemic areas by means of annual mass drug administration (MDA) for 4–6 years [7]. The Bangladesh government for its part made a national plan to eliminate the disease by 2015 [8]. Since the start of GPELF, many endemic countries have completed the planned MDAs and the prevalence of filarial infection has been reduced significantly [9–13]. At this point of post-MDA low endemic stage, these countries have to formulate the 'end-game' strategies in which the methods and criteria to stop MDAs, confirm elimination and detect resurgence need to be addressed [14–17].

In post-MDA stage, when microfilaria (mf) densities have become low, the standard blood films are not sensitive anymore, and immunodiagnoses that detect filarial antigens or specific antibodies will be able to play a more significant role. Itoh et al. (2001) reported an ELISA that uses urine samples for serum. It showed a high sensitivity of 95.6% with *Wuchereria bancrofti*-infected Sri Lankans and a specificity of 99.0% with urine from non-endemic areas in Laos, Thailand, and Japan [18]. Due to its ease in collecting samples, the ELISA has been accepted well by the people living in endemic areas.

We planned to apply the urine-based ELISA method in Bangladesh and as a first step, evaluated the sensitivity and specificity with Bangladeshi urine samples, and then conducted a prevalence survey with schoolchildren in a post-MDA area. The result was compared with the prevalence obtained by ICT antigen test.

2. Materials and methods

2.1. Study area and subjects

2.1.1. Sensitivity study

In Ranishankail and Haripur upazilas (sub-districts) of Thakurgaon district in the northern region of Bangladesh where lymphatic filariasis is endemic and 4 rounds of MDA have been completed, 749 people (680

* Corresponding author. Tel.: +81 561 62 3311; fax: +81 561 63 3645.
E-mail addresses: sohelsamad_84@yahoo.com (M.S. Samad), macitoh@aichi-med-u.ac.jp (M. Itoh), moji-k@chikyu.ac.jp (K. Moji), profdrmoazzemhossain@gmail.com (M. Hossain), din63d@icddrb.org (D. Mondal), shafiul@icddrb.org (M.S. Alam), kimura@aichi-med-u.ac.jp (E. Kimura).

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males, 69 females) aged between 20 and 70 years (mean: 34 years), were examined for the circulating filaria antigen with immunochromatographic card test (NOW®ICT, Binax Inc., Portland, Maine, USA). After obtaining the results, urine samples were collected from 105 ICT positive people. On the same day at night (10:00 pm–12:00 midnight) blood samples were collected from them for mf smears.

2.1.2. Specificity study

Dagonbhuiyan upazila of lymphatic filariasis non-endemic Feni district in the southern part of the country was selected. A total of 104 males aged 20 to 70 years were selected as non-endemic healthy controls (NEHC) and examined with ICT for the confirmation of antigen negativity, and their urine samples were collected to be used as negative standard.

2.1.3. Prevalence study

The efficacy of detecting infection/exposure to *W. bancrofti* was compared between urine ELISA and ICT with 319 schoolchildren aged 5–10 years in Atowari upazila, Panchagarh, the northernmost district of Bangladesh. The upazila showed the low mf prevalence rate (1.4%) after 5 rounds of MDAs (2008, government report).

2.2. Specimens

For all blood tests, finger-prick blood was used: 100 μl for ICT, and 60 μl for night blood smears. For urine ELISA, 5 ml urine was collected, added with sodium azide at the final concentration of 0.1% as a preservative, and the samples were stored at 4 °C except during the period of transportation to Japan.

2.3. Ethical considerations

The participants were informed about the purpose of the study and that their samples would be transported to Japan for research purposes only, and not for the individual diagnosis of the disease. Prior to the collection of all samples, the written informed consent from all the participants or from their headmasters/mistresses in the case of minor school children were obtained. The study was reviewed and approved by the Ethics Committee of Aichi Medical University School of Medicine and the National Research Ethics Committee (NREC) of the Bangladesh Medical Research Council.

2.4. Enzyme-linked immunosorbent assay

The urine ELISA developed in our lab that detects filaria specific IgG4 from urine samples [18] was performed with a modification [19]. In brief, flat bottomed, 96-well microtiter plates (Maxisorp™; Nunc, Roskilde, Denmark) were coated with recombinant Wb-SXP1 antigen (recWb-SXP1) (1 μg/ml) at 4 °C overnight. After blocking with the casein buffer (1% casein in 0.05 M Tris–HCl buffer with 0.15 M NaCl, pH 7.6) for 2 h at room temperature, urine samples were directly applied to the plates (100 μL per well) and incubated overnight at 25 °C. After four washes with phosphate-buffered saline (PBS), pH 7.4 containing 0.05% Tween 20, 100 μL peroxidase conjugated mouse monoclonal antibody to human IgG4 (Southern Biotech, Birmingham, AL), diluted 1: 4000, was added to each well. After incubation at 37 °C for 1 h, the plates were washed four times, and then incubated with ABTS® Peroxidase Substrate System (KPL Inc., Gaithersburg, MD) for one hour at room temperature and the optical density was measured at 415 nm and 492 nm as a reference. Each sample was assayed in duplicate. Antibody levels were expressed as units (U) estimated from a standard curve constructed with serially diluted positive sera ranging from 0 to 7290 U. The cutoff value in the study was 7.08 U.

3. Results

3.1. Sensitivity studies

For the sensitivity study, 749 people were screened first with ICT, and 105 positive subjects were found. Then, the positives were tested for mf and 31 positives and 74 negatives were obtained. On this basis, 2 categories of positive standards were considered: positive standard 1 (PS1); 31 subjects with both ICT and mf positives (Ag+/mf+) and positive standard 2 (PS2); 105 ICT positives (Ag+/mf + and Ag+/mf−). With PS1 and PS2, the sensitivity of urine ELISA was found to be 96.8% (30/31) and 84.4% (89/105) respectively (Table 1). Out of the 16 ELISA negatives 15 were mf negative as well (Fig. 1). There were no differences in the average antibody units (1017 versus 1015 U; *t*-test, $P > 0.9$) between Ag+/mf + and Ag+/mf− people.

3.2. Specificity studies

All of the selected 104 subjects from the non-endemic area were tested with ICT, confirmed to be negative and then used as the NEHC. Their urine samples were all negative with ELISA resulting in 100% specificity. The mean antibody titer among the NEHC was found to be very low (Fig. 1).

3.3. Prevalence study

With 319 schoolchildren between the age group of 5 years to 10 years, the ELISA detected 7 positives (2.2%), while ICT did only 1 (0.3%) (Table 2). However, the difference was not statistically quite significant (χ^2 test with Yate's correction, $P = 0.075$) and the ICT positive subject was ELISA negative. The IgG4 titers are analyzed according to age (Fig. 2). There was no positive among 5 year to 6 year old children (0/102) and the age of the youngest IgG4 positive was 7 years. The positive rates of age group 7 years, 9 years and 10 years were 1.6% (1/62), 4% (2/49) and 6.8% (4/59) respectively. This shows a trend of gradual increase in the number of IgG4 positives and IgG4 titers with age, although significance analysis could not be done because of few numbers of positives. The highest IgG4 titer among the positives was found to be 52.3 U, indicating a low level of antibody which is considered normal in an area where five rounds of MDA have already been completed. In addition, the distribution of titers under the cutoff line shows a clear dot-sparse space.

4. Discussion

In the present study in Bangladesh, with the most certain positive standard, that is, mf and ICT positives, urine ELISA with recWb-SXP1 resulted in 96.8% sensitivity. With a more practical positive standard (ICT positive subjects), the ELISA gave 84.8% sensitivity, which was considered satisfactory for use in Bangladesh field. The sensitivity seems to be lower than the 95.6% sensitivity reported in Sri Lanka with Og4C3 antigen test (Trop-Ag *W. Bancrofti*, TropBio Pty. Ltd., Queensland, Australia) positives as the standard [18]. The difference could be explained in part by the fact that Sri Lankan data were obtained before MDAs, while ours were after 4 rounds of MDA which significantly

Table 1
Sensitivity of the urine ELISA in Bangladesh with 2 different positive standards (PS1 and PS2).

Category of positive standard	No. of subjects examined	Urine ELISA	Sensitivity (%)
		No. of positives	
PS1: Ag (+) and mf (+)	31	30	96.8
PS2: Ag (+) and mf (+ & −)	105	89	84.8