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SHORT COMMUNICATION

Scope of detectability of circulating antigens of human lymphatic filarial parasite *Wuchereria bancrofti* with smaller amount of serum by Og4C3 assay: its application in lymphatic filariasis elimination programme

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Abstract Filarial antigen detection is an appropriate epidemiological indicator for mapping lymphatic filariasis and impact evaluation of filariasis elimination programme in view of low sensitivity of parasite detection. Monoclonal antibody-based Og4C3 immunological test requires 100 µl serum, which is difficult to collect by finger prick method during community based surveys. Hence, we tested lesser volume of serum compared to standard volume of 100 µl to compare its sensitivity and specificity in detecting the circulating filarial antigens. Blood samples were collected from individuals who tested positive [with titer groups 4 (border line positives), 6 (medium positives), and 8 (high positives)] and negative (titre group 3) for Og4C3 assay. Different volumes of serum samples were used to make-up required volume (100 µl) with appropriate dilutions and subjected to Og4C3 assay. The results showed that known negative samples tested negative at all the serum volumes tested. All positives (titer groups 6 and 8) showed positivity at all reduced volumes of serum sample. However one of the medium positive sample showed negative reaction in 5 µl volume of serum and two of the border line positives showed negative at all the serum volume tested. The results thus showed as less as 15 µl serum is adequate for use in Og4C3 assay. So the test can be performed without losing its sensitivity even with 5 µl serum samples at high titre of antigen (titre group 8) and 15 µl for other groups and this method has scope in programme evaluation.

Keywords *Wuchereria bancrofti* · Antigens · Og4C3 assay · Serum volume · Transmission

Introduction

Lymphatic filariasis (LF), a disabling disease caused by the parasite *Wuchereria bancrofti* is prevalent in tropical and subtropical countries and an estimate of 120 million people are affected in 73 endemic countries (World Health Organization 2012). In India, LF is endemic in 20 states with 610 million people residing in endemic areas (Raju et al. 2010; World Health Organization 2012) The Global Programme to Eliminate Lymphatic Filariasis (GPELF) was launched in 1997 and over 570 million people are covered under this programme in 48 countries with indigenous transmission (Ottesen et al. 2008).

Mass drug administration (MDA) with annual single dose of DEC co-administrated with albendazole for 5–6 years and distribution of mass DEC fortified salt are the two recommended strategies towards filariasis elimination (Ottesen et al. 2007). Mapping, monitoring and evaluation are inbuilt components of the programme. Appropriate epidemiological indicators with high sensitivity and specificity are required for these activities, particularly in generating data for making evidence based decisions.

The diagnosis of LF based on the microscopic examination of finger prick thick blood smear for microfilaraemia has been in operational use, but is less sensitive for active infection as this technique misses people with low microfilaria counts and those with amicrofilaremic infections who have the potential to contribute to future

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transmission (Weil 2005). Further, it is constrained by collection of blood smears during night time. Immunological tests, based on monoclonal antibodies against filarial parasite antigens, have been developed in the past and are available commercially. Og4C3 ELISA is one such assay (More and Copeman 1990) and is widely used by researchers for detecting circulating filarial antigens. Considering the high sensitivity and specificity (Simonsen and Dunyo 1996; Gyapong et al. 1998) and also the advantage of using these kits with day blood samples, utility of these immunological markers (Lammie et al. 2004; Ramzy et al. 2006; Helmy et al. 2006; Weil et al. 2008) in antigen detection has potential application in detecting the foci of infection as well as evaluating the impact of transmission interruptions. But a minimum of 100 µl serum is required to perform this test as per the manufacturer's recommended procedure which is one of the limitations while drawing the sample by finger prick method. Further, large quantity of diluted test serum sample remains unutilized as the serum sample is diluted to 1:3 ratio (100 µl of serum + 300 µl sample diluent) and from this only 50 µl diluent is used for the assay. In this process 350 µl of diluted serum is left without any use except in repeated tests.

While collecting finger prick blood samples, difficulties are encountered in drawing at least 250 µl of blood to obtain 100 µl of serum. Therefore, samples with less than 100 µl serum remain unprocessed and hence warrants modifications in the procedure without compromising the sensitivity of the assay. Hence without compromising the result we wanted to know the utilization of lower amount of serum in detecting the positivity. In this context, we carried out a study with varying volumes of serum, lesser than the standard volume, but without changing the dilution factor to assess the sensitivity and specificity of the Og4C3 assay and the results are discussed in this communication.

Ethics

We the authors of the manuscript state that the protocol for the research project has been approved by the Scientific Advisory Committee (SAC) of the institute. The Human Ethical Committee (HEC) of our centre (Vector Control Research Centre) approved the project for collecting the required blood from the individuals. Informed consent forms with the details of the project and the possible risks and benefits of the project has been issued to the volunteers before collecting the blood samples.

Materials and methods

Blood samples were collected during day time by finger prick method. Out of 12 samples collected 3 were from the antigen negative individuals and 9 from antigen positive individuals based on the antigen test performed earlier with these samples and the serum separated. These positive samples were further grouped into various titre groups (TG) such as high positives (TG 8), medium positives (TG 6) and border line positives (TG 4). The allocation of TG were based on the optical density (OD) values and the corresponding antigen units as given in the instruction manual. The interpretation of the results are given in the end of the materials and method section.

Informed consent was obtained from all the study individuals. Detection of filarial specific antigen in the sera samples were carried out using TropBio ELISA kit (catalogue No 03-010-01) as per the instructions of the manufacturer (JCU Tropical Biotechnology Pvt. Ltd., Queens Land, Australia). ELISA kit for detecting and quantifying *W. bancrofti* antigens were procured from TropBio Pvt. Ltd., Australia. The 96 well microtitre plate supplied were coated with a monoclonal antibody (Og4C3) specific to *W. bancrofti* antigen in human sera. Seven dilutions of the standard antigens were also supplied along the kit and were used as per the plate layout diagram. As per the procedure 100 µl of test serum was added to 300 µl of sample diluent. In addition to the recommended 100 µl serum, serum quantities such as 15, 25 and 50 µl were used in the same dilution ratio of 1:3 to make-up final volume of 60, 100 and 200 µl respectively and were tested. We also tested 5 µl serum but at a dilution ratio of 1:20 (5 µl serum + 95 µl sample diluents) to make a 100 µl volume. After adding the diluted serum samples, plates were incubated at 37 °C for 1.5 h. After washing the plate, diluted rabbit anti-*Onchocerca* antibody was added to the wells and incubated for 1 h. Plates were washed again and diluted anti-rabbit horse raddish peroxidase conjugate was added and incubated for 1 h. After the final wash plates were added with the ABTS substrate and incubated for 1 h. Then the OD of the wells were read at 414 nm in a microplate spectrophotometer (SPECTRAmax plus^{384}, Sunnyvales—CA, USA) by blanking the wells having sample diluent. Seven dilutions of the standard antigens provided in the kit were used to decide the cut off value for positivity and negativity of the samples. Using the seven standards the test samples were allocated into eight TG. Titre group 1 and 2 are definitely considered as negatives as their antigen units are <10 and 32 respectively. Titre group 3 is considered as equivocal or suspect reactors (antigen units is 128). Titre group 4 to Titre group 8 has the antigen units of 512–32,000. The OD values of samples equal or more than the titre group 4 (standard no.: 4–512 antigen units) were considered as positive, as per the manufacturer's guidelines.

Table 1 Positivity and negativity of samples tested with different quantity of serum for filarial specific antigens by Og4C3 antigen assay

Category (titre group—TG)	Subject no.	Test result at base		OD value with reactivity (+ve/−ve) at different volume of sample				
		OD value	+ve/−ve	5 µl	15 µl	25 µl	50 µl	100 µl
3 (Negatives)	1	0.245	−ve	0.007 (−ve and TG 3)	0.026 (−ve and TG 3)	0.016 (−ve and TG 3)	0.03 (−ve and TG 3)	0.008 (−ve and TG 3)
	2	0.019	−ve	0.015 (−ve and TG 3)	0.017 (−ve and TG 3)	0.034 (−ve and TG 3)	0.001 (−ve and TG 3)	0.022 (−ve and TG 3)
	3	0.083	−ve	0.028 (−ve and TG 3)	0.31 (−ve and TG 3)	0.167 (−ve and TG 3)	0.374 (−ve and TG 3)	0.244 (−ve and TG 3)
4 (Border line positives)	1	0.398	+ve	0.005 (−ve and TG 3)	0.006 (−ve and TG 3)	0.011 (−ve and TG 3)	0.001 (−ve and TG 3)	0.001 (−ve and TG 3)
	2	0.828	+ve	0.063 (−ve and TG 3)	0.015 (−ve and TG 3)	0.016 (−ve and TG 3)	0 (−ve and TG 3)	0.006 (−ve and TG 3)
	3	0.496	+ve	0.246 (−ve and TG 3)	1.274 (+ve and TG 4)	1.615 (+ve and TG 4)	1.539 (+ve and TG 4)	1.617 (+ve and TG 4)
6 (Medium positives)	1	1.869	+ve	2.211 (+ve and TG 6)	2.313 (+ve and TG 6)	2.378 (+ve and TG 6)	2.406 (+ve and TG 6)	2.399 (+ve and TG 6)
	2	1.936	+ve	1.979 (+ve and TG 6)	2.139 (+ve and TG 6)	2.163 (+ve and TG 6)	2.286 (+ve and TG 6)	2.286 (+ve and TG 6)
	3	1.285	+ve	0.993 (−ve and TG 3)	1.692 (+ve and TG 6)	1.983 (+ve and TG 6)	1.858 (+ve and TG 6)	1.988 (+ve and TG 6)
8 (High positives)	1	2.083	+ve	2.327 (+ve and TG 8)	2.354 (+ve and TG 8)	2.422 (+ve and TG 8)	2.443 (+ve and TG 8)	2.425 (+ve and TG 8)
	2	2.145	+ve	2.244 (+ve and TG 8)	2.333 (+ve and TG 8)	2.346 (+ve and TG 8)	2.376 (+ve and TG 8)	2.375 (+ve and TG 8)
	3	2.206	+ve	1.174 (+ve and TG 8)	2.025 (+ve and TG 8)	2.279 (+ve and TG 8)	2.136 (+ve and TG 8)	2.209 (+ve and TG 8)

Results

Details of TG, reactivity of different volumes of samples in Og4C3 ELISA are presented in Table 1. All the serum samples from high titre group individuals (TG 8) with different volumes showed positive reaction, with OD values found to range between 1.174 and 2.443 and were above the cut off OD value of 1.115. This group of individuals tested positive even at 1:20 dilution using 5 µl serum (OD value 1.174–2.327, qualified as high positive).

Samples collected from individuals in the medium titre group (TG 6), also showed positive reaction. All test samples with different volumes of serum showed high positive reactivity with the OD values >2.0, except 5 µl volume of sample at the dilution of 1:20. In case of border line titre group (TG 4), samples from two out of three individuals showed negative when tested with different test sample volumes (5–100 µl). Sample from one individual showed positive reaction in all the dilutions except at 5 µl. This indicates that 20 times dilution of 5 µl did not show positive reaction in medium and low positive samples.

The specificity and the sensitivity of the assay performed by us with varying lesser volumes of serum was found to be 100 and 71.4 %. The lesser sensitivity is due to two TG4 positive samples which were detected to be negatives at 5 µl dilution and the reason could be that, the antigen units are just at border level in the above negative samples.

Discussion

Appropriate epidemiological markers are necessary for situation analysis as well as for assessing the impact of interventions against public health problems. The first step in undertaking large scale programme to eliminate LF is to delimit the (mapping) the areas for the intervention. Antigenemia or microfilaria surveys are recommended (World Health Organization 2000, 2005) for mapping and the areas with $\geq$1 % mf prevalence are included for Programme to Eliminate Lymphatic Filariasis (PELF). Using these criteria, over 1.3 billion people in 81 endemic countries have been estimated to be at risk (World Health Organization 2009) of filarial infection. Antigen prevalence in children in the age group of 6–7 years is recommended (World Health Organization 2011) to verify absence of transmission, as an indicator to assess the impact of MDA towards PELF. The life span of the adult worm was 10.2 years without chemotherapy and it was reduced to 5.3 years following diethyl-carbamazine therapy (Vanamail et al. 1990). Another study reports that the adult parasites can live as long as 10 years in humans (Subramanian et al. 2004) and samples from older age class would show positive reaction making it difficult to

decide whether the infection occurred during the intervention period or earlier. Therefore, it is ideal to screen children who were born after the introduction of MDA to verify whether transmission was interrupted or not. Also, for individual case detection and treatment antigen test will be advantageous over mf and hence antigen tests assume importance in the LF control. Recently we reported a protocol to carry out a battery of tests in monitoring several immunological and parasitological and molecular parameters (Hoti et al. 2008). Antigen tests are more sensitive (Lammie et al. 1994) and are used to assess the situation (Bal et al. 2009; Das et al. 2005; Melrose et al. 2000; Nuchprayoon et al. 2001; Shah and Mulla 2007; Steel and Ottesen 2001; Tisch et al. 2001; Weerasooriya et al. 2002) in different endemic countries. Antigen tests were also used to evaluate other methods of detection (Rocha et al. 1996; Wattal et al. 2007). Considering the non-endemic normals and microfilaria carriers, the Og4C3-ELISA test was found to have 100 % sensitivity and 94.12 % specificity for detection of Mf carriers in sera samples. Comparison of finger prick dried blood spots (DBS) and sera samples by ELISA vis-à-vis the immunochromatographic test (ICT), carried out on Mf carriers ($n = 91$) and endemic normals ($n = 97$), showed a positivity of 88 (96.7 %) in DBS as against 86 (94.5 %) in sera samples and 88 (96.7 %) by ICT, amongst Mf carriers, with a statistically significant correlation in antigen units between sera and DBS samples ($r = 0.959$, $p = 0.000$) amongst the microfilaria carriers (Rocha et al. 1996). But it is cost prohibitive for field use. Also, tests such as ELISA based Og4C3 test kits require at least 250 µl of blood (which yields 100 µl serum) which may not be always possible by finger prick method to collect from every individual in field surveys. Hence, we standardized the procedure with blood samples of 50 µl (which yields 20 µl of serum) much lesser than 250 µl. However this method can be tested in a multicentric level before applying this test in evaluating the intervention programme at national level. This will make antigen evaluation more feasible with the scope of its application in evaluating the programme to eliminate LF. Filter paper compatible Og4C3 test procedures are also available (Gyapong et al. 1998) which makes it more user friendly.

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