FULL LENGTH RESEARCH ARTICLE

SENSITIVITY OF SOME IMMUNOGLOBULIN G CLASS AND SUBCLASS ANTIBODIES TO ADULT *Onchocerca volvulus* SDS-EXTRACTED ANTIGENS.

*OSUE, H. O[1].; GALADIMA, M[2].; ODAMA, L. E[2], EDEGHERE, H. U[1]. & ENGELBRECHT, F.[3]

[1]*Nigerian Institute for Trypanosomiasis Research*
P.M.B. 2077, Kaduna, Nigeria
[2]*Department of Microbiology*
Ahmadu Bello University, Zaria, Nigeria
[3]*Hygeuine Institue, Abt. Parasitologie Im Neunheimer Feld 32469120 Heiderbeg, Germany*
*(Corresponding author)
osueho@yahoo.com

ABSTRACT

Indirect sandwich enzyme-linked immunosorbent assay (ELISA) was used to measure serum antibody responses in onchocerciasis patients. Apparently, IgG antibody class was more sensitive than IgG1, IgG3 and IgG4 responses to *Onchocerca volvulus* adult worms sodium duodecyl sulphate (SDS) extracted crude antigens in proven clinical and parasitological cases (n=95). Sensitivity varied slightly among the IgG1, IgG3 and IgG4 isotypes. All cases were positive for IgG, with 98%, 97% and 96% for the IgG isotypes respectively. Those with evidences of palpable nodule and skin microfilariae (n=32) were all seropositive (100%) for the three IgG isotypes-assays. A decrease in seropositivity was recorded among the assumed endemic normals or those with no evidence of infection (n=19), yet IgG recorded the highest with 90% and IgG4 was the least with 58%. It is concluded that the IgG1 and IgG3 assays have great potential as a screening test, while IgG4 could serve as a confirmatory test. These assays could be useful in detecting cases elusive to parasitological and clinical prognosis particularly in post-control surveillance situations.

Keywords: *Onchocerca volvulus,* Serology, sensitivity, Screening, Diagnosis, Antigens and Antibodies.

INTRODUCTION

Definitive diagnosis of onchocrciasis is based on the detection of microfilariae in skin snip biopsies of the patients. This method is more sensitive than the use of rapid assessment, which depends on palpation of nodules and presence or absence of depigmentation of skin (Edungbola *et al*. 1990; Taylor *et al*. 1989).

To some extent, individuals with prepatent infection and low microfilarial load (MFL), particularly in an area with on-going control programme will be misdiagnosed. In addition to this, skin snipping carries the risk of transmitting bloodborne viruses such as hepatitis virus and human immunodeficiency virus (HIV) (WHO 1987; Ogunriade *et al.* 1993). Current mass distribution of ivermectin to the needy endemic communities could bring the disease to a level requiring active case detection and treatment. The implementation of an effective control strategy will therefore rely on reliable, simple and affordable diagnostic method, particularly as the most likely cause of onchocerciasis recrudescence will be by importation of the parasite through human immigration and fly invasion of areas where the disease is not normally a problem (Habbema *et al.* 1992).

The use of clinical prognosis, antigen, and DNA has limitations because of insensitivity; circulating antigens form complexes with antibodies, and the hazard of working with radioactive substances. Lately, polymerase chain reaction (PCR) was used to amplify the DNA probe and subsequently detected by enzyme-linked immunosorbent assay (Zimmerman *et al*. 1994) thereby eliminating the use of radioisotope. Yet, field applicability, availability, and cost of reagents and the need for skin snipping make the approach not likely to gain wide acceptance for primary surveillance. A viable option being considered is the antibody detection assays.

The use of monoclonal antibody, affinity purified or recombinant antigens and IgG4 isotype assays has eliminated the main problem of cross-reactivity involving phosphorylcholine molecule (Ottesen *et al*. 1985; Lal & Ottesen, 1988; Weil *et al*. 1990; Chandrashekar *et al.* 1996). The ubiquitous molecule is found in bacteria, fungi, and other parasite species. The SDS extract contain mostly low molecular weight antigens reported to be more species specific (Weiss & Karam, 1987; Engelbrecht *et al*. 1991). With this development, a remarkable improvement in the reliability of serodiagnosis of the disease was achieved. This study was aimed at assessing the usefulness of sodium duodecyl sulphate (SDS) salt extracted antigens of the adult worm *O. volvulus* for sero-diagnosis. Sensitivity of IgG1, IgG3 and IgG4 responses to the antigens in individuals from endemic area were measured in indirect enzyme-linked immunosorbent assay (ELISA). Subjects who were screened comprised of individuals with different infection status; presence or absence of palpable nodule and skin microfilaria, with or without evidence of onchocercal related clinical manifestations.

MATERIALS AND METHODS

Experimental design and sample collection: The study area and population from where the samples were obtained has been described (Osue 1996). Subjects were selected based on evidence of one or more clinical presentations. Thereafter, the skin snip results were obtained. The sample population (n=114) was divided into microfilarial positive (n=62) and skin mf negative (n=52) groups. The mf positive group was further divided into palpable nodule positive (n=32) and palpable nodule negative(n=30) sub-groups. Microfilarial negative individuals were divided into palpable nodule positive subgroup (n=18) and negative subgroup with clinical evidence (n=15). Those with no

clinical signs of the disease (n=19) were tentatively referred to as endemic normal (EN) or putatively immune individuals. Blood samples were collected by venous puncture from freely consenting individuals. Serum was extracted from the blood after allowing clotting for 2 hrs at ambient temperature. Serum samples were aliquot in 30µl per well of microtitre plate and frozen in deep freezer and thawed once just before use.

Parasite antigens: The crude SDS extracted antigens from adult *O. volvulus* was prepared as described by Engelbrecht *et al*. (1991).

Serology: Serum antibody reactivity with the antigens was tested in sandwich ELISA. The IgG1, IgG3 and IgG4 antibodies were measured using a modified protocol (Engelbrecht *et al.* 1992) that involved coating microtitre plates with antigens diluted in carbonate buffer (pH9.6) at 1:1000 and incubated overnight at 4°C. All other steps were performed at room temperature (RT°C). The unspecific sites were blocked with 200µl per well of 1-2% bovine serum albumin (BSA) for 1hr. Serum was added at 1:500 for IgG1, 1:100 for IgG3, 1:200 for IgG4. Thereafter, monoclonal antibodies obtained from Sigma specific for each isotype, IgG1 (clone HP-6001, 1:2000), IgG3 (HP-6050, 1:8000) and IgG4 (HP-6025, 1:8000) was added at 150µl per well. It was followed by goat anti-mouse IgG (H+L) horseradish peroxidase conjugate (BIORAD) at 1:1000 dilution.

Antigen and antibody reactions were detected by addition of freshly prepared substrate solution containing 200µl orthophenylene diamine (OPD) (from Sigma) in 20µl hydrogen peroxide, 0.1M citric acid and 0.2M Na_2HPO_4 buffer and allowed reacting for 15 minutes. The enzyme reaction was terminated with 30µl per well of 2M H_2SO_4. Optical densities (OD) of wells of microtitre plates were measured in a Dynatech ELISA reader (model MR4000) at 492nm test and 630nm reference filters.

Assay control: Each assay included a negative control and a positive standard (in 5 wells per plate) and they had been selected from among the serum samples in pre-titration experiments. In addition, there were four internal control blank wells (without serum) per replicate plate. Optimum concentration of antigens, serum, monoclonal antibodies, and conjugates were determined in a series of preceding titration experiments. The final assays were performed in duplicate. Cut-off points were calculated as mean plus two standard deviations (SD) for non-endemic controls (n=7).

RESULTS

Serodiagnostic sensitivity of antibody responses to antigens: Serum antibody reactions with crude extract of the adult worm of *O. volvulus* were measured using indirect ELISA. An individual whose OD-value was above the cut-off points (mean+2SD of non-endemic controls) (n=7) was regarded to be positive. Sero-positivity only varied slightly among IgG1, IgG3 and IgG4 isotypes. A sample population (n=95) with proven parasitological and or clinical evidence of infection were sero-positives for IgG1, IgG3 and IgG4 with 98%, 97% and 96% respectively (Table1). Furthermore, the three isotypes were equally sensitive (100%) in individuals having palpable nodules and skin microfilariae (N+ mf+)(n=32). However, a drop in sensitivity of IgG3 and IgG4 to about 87% and 80% respectively was recorded in nodule and microfilarial negative subgroups (n=15) as shown on Table 2. Among the endemic normal (EN) individuals (n=19) both IgG1 and IgG3 isotype antibodies showed 79% as against that of IgG4 with 58%. But within the non-endemic control, both IgG3 and IgG4 had a case each (in different individual) of very weak sero-positivity with 0.17 and 0.15 OD-value respectively.

Sensitivity of assay at higher cut-off point: Sensitivity of the assays analysed with the cut-off points increased from the mean plus2SD to 3 SD. This resulted in decrease sensitivity. The highest decrease was recorded for IgG3 with 88.7% and slightly for IgG4 and IgG1 with 95.2% and 98.4% respectively. In the skin mf negative group (n=33) IgG3 and IgG4 were both 87.9% while IgG1 had 81.8%. The sensitivity of IgG3 was 68.4% and IgG1 had 63.2% in the endemic normal (n=19) and IgG4 remained unchanged at 58%.

DISCUSSION

Results from this study have demonstrated the higher sensitivity of IgG1, IgG3 and IgG4 antibodies to crude antigen. Emphasis has been on exploring the antigenic distinction of IgG4 reported to be more species-specific (Ottesen *et al.* 1985; Lal & Ottesen, 1988; Weiss & Karam, 1989; Weil *et al.* 1990; Engelbrecht *et al.* 1992) have

TABLE 1: SENSITIVITY OF ANTIBODIES TO *Onchocerca volvulus* EXTRACT IN ENDEMIC NORMALS (EN) COMPARED TO POSITIVE CASES.

Infection status	IgG	IgA	IgM	IgG1	IgG3	IgG4
Cut-off values*	0.19	0.07	0.34	0.14	0.16	0.14
EN (n=19)	17	9	6	15	15	11
Sero+ve (%)	89.5	47.4	31.6	79	79	57.8
Cases (n=95)	95	73	29	93	92	91
Sero+ve (%)	100	76.8	30.5	97.9	96.8	95.8

*Computed mean plus two (2) standard deviations of optical density (OD) values of non-endemic controls (n=7

TABLE 2: SENSITIVITY OF ANTIBODIES TO *O. volvulus* EXTRACT DEPENDING ON PARASITOLOGICAL STATUS.

Infection status	IgA	IgM	IgG1	IgG3	IgG4
N-mf- (n=15)	13	2	14	13	12
Sero+ve (%)	86.7	13.3	93.3	86.7	80.0
mf-(n=18)	15	3	17	18	17
Sero+ve (%)	83.3	16.7	94.4	100	94.4
N-mf+ (n=30)	18	11	30	29	29
Sero+ve (%)	60.0	36.7	100	96.7	96.7
N+mf+(n=32)	26	13	32	32	32
Sero+ve (%)	81.3	40.6	100	100	100

engendered very high assay specificity without compromising assay sensitivity. The performances of these assays were analyzed using the presence or absence of palpable nodule and or skin microfilariae. The very high sensitivity of IgG1, and IgG3 and IgG4 responses to the parasite extract seems assuring that these tests could be useful in serodiagnosis of onchocerciasis. For any of these assays to be acceptable, its specificity should be comparably high. The detergent SDS-extract may have advantage over water-soluble or PBS-extract hitherto used for antibodies detection tests. Earlier qualitative analyses of SDS-extract prepared from an animal infective species; *O. gibsoni* (Cabrera *et al*. 1986) was reported to contain fewer high molecular weight antigens than the water-soluble extract. A similar observation had been made for *O. volvulus* extract (Engelbrecht *et al*. 1991). The species-specificity of IgG4 antibody to low molecular weight antigens is well documented (Weiss & Karam, 1989; Bradley *et al*. 1998). This raised the great expectation for the reliability of IgG4 assay in particular followed by IgG3 and IgG1 ability to discriminate between infection statuses.

Available reports (Lucius *et al*. 1992; Ogunrinade *et al*. 1993) showed that in IgG4 assays using recombinant and monoclonal antibody defined antigens, up to 95% sensitivity and 98% specificity were recorded which compared favourably well with the overall 96% recorded here. Although defined antigen may have an edge over crude antigen, the technique of preparing the former may not be readily available in endemic areas. Therefore, the use of the animal infective species, *O. gibsoni* could guarantee large supplies of adult worm. In this case the problem of variation from one batch preparation to another will have to be tackled.

A remarkable similarity between IgG3 and IgG4 qualitative response to the crude extract in immunoblot assays has been demonstrated by Cabrera *et al*. (1986) and Engelbrecht *et al*. (1991). From our result, the reliability of the observed little difference between the two isotypes using quantitative test appears promising for serodiagnosis. When compared with the cocktail recombinant protein antigens that showed an impressive 98% sensitivity and 100% specificity in IgG4 assay (Bradley *et al*. 1998; Vincent *et al*. 2000), the 98% sensitivity among the skin mf positive individuals (n=62) used in this study is highly commendable.

Furthermore, whether this and the low rate of IgG4 sero-positives among the endemic normals (n=19) is a demonstration of superiority in assay specificity over IgG3 has to verified. It has been speculated that because IgG4 antibody is produced in response to chronic stimulation, might limit its usefulness in children (Bradley *et al*. 1993). Conversely, IgG1 and IgG3 will likely be detected much early during infection. By analogy, its higher sero-positive rate among the mf negative group (n=33) than the mf positive group (n=62) support the above assertion. It has been suggested that detection of prepatent infections (especially in children) and convalescence cases in adult, IgG1 or IgG3 stand to complement IgG4 as a screening and diagnostic test respectively. Moreover, consequent to treatment, IgG4 has been known to decrease much latter than the others (Gbakina *et al*. 1992). Despite the decline in sensitivity when higher cut-off point (mean+3SD) was used; the fact remain, one test will detect cases missed by the other. Only when this extract is able to eliminate the problem of unspecificity associated with detection of antibodies can the recorded high sensitivity be meaningful. The level of anti-filarial antibody responses in subjects having no palpable nodule and skin microfilaria (n=15) and in assumed endemic normal individuals show they have been exposed to infection. A general set back of antibody test is that it cannot confirm whether they were carrying active infection or not.

This study has been demonstrated that IgG1 or IgG3 and IgG4 reaction with SDS extract from adult worm *O. volvulus* native antigens is a potential tool for sero-diagnosis of onchocerciasis. The assays may be adapted for screening and confirmation tests respectively and for surveillance during operational research for post-control monitoring or for routine laboratory test. It may prove more useful particularly in detecting cases elusive to parasitological and clinical prognosis. Further work is required to assess the ability of these assays to discriminate onchocerciasis from other related and unrelated parasitic and bacterial infections.

ACKNOWLEDGEMENT

We thank the Director-General of the Nigerian Institute for Trypanosomiasis Research for funding this project. We appreciate the major input from European Economic Community (EEC) project grant TS3*-CT92-0057 to Dr. H.U. Edeghere (NOCP, Kaduna, Nigeria). We are grateful to all the officials of Kachia Local Government Area, Kaduna State and the villagers who volunteered to participate in the project.

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