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Full Length Research Paper

Antibody responses to a recombinant *Onchocerca volvulus* antigen (Ov1.9) by onchocerciasis patients before and two months after Ivermectin treatment

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Indirect sandwich enzyme-linked immunosorbent assay (ELISA) was used to measure serum IgG antibody responses to Onchocerca volvulus designated (Ov1.9) recombinant antigen in onchocerciasis patients (n=114) prior to initial ivermectin treatment. Paired sera (n=32) obtained 2months after treatment were also analyzed for changes in antibody levels in a sub-population at pre- and post-treatment. The difference between mean optical density (OD) values of IgG antibody level in proven cases (n=95) and the assumed endemic-normal (EN, n=19) of 0.58±0.23 versus 0.37±0.21, respectively was statistically significant (P<0.05) by the t-test of unpaired data. The non-endemic (NEN, n=7) control had mean OD value of 0.12±0.02. There was no significant difference between the sexes or among age groups and the infection subgroups (with or without presence of nodule and/or skin microfilariae mf). At a cut-off point of mean plus 2 standard deviations of OD value for the NEN control, the sensitivity of 87% in proven cases of onchocerciasis (n=95) and 89% in those with skin mf positive (n=62) proved that Ov1.9 may be suitable not as a single screening test reagent, but when in cocktail with one or more antigens. The higher mean OD value in those with chronic skin disease than those without clinical signs of onchocerciasis (n=39); with papular onchodermatitis (n=20) and optic nerve disease (n=12) subgroups was statistically significant by t-test of unpaired data (P<0.05). The heightened IgG antibody response to Ov1.9 with chronic skin disease is clearly indicative of the possible role parasite-specific antibodies may play in the immunopathology of the disease. There was no significant increase in mean antibody level two month post-ivermectin treatment; hence the antigen will not be useful for drug screening.

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

INTRODUCTION

Onchocerciasis is transmitted through the bite of a ferocious tiny black fly vector, *Simulium damnosum* complex. The microfilaria are either ingested or released in the process. Intact adult worms are encapsulated in subcutaneous nodules particularly on bony prominences as a result of body reaction to the worm and some adult worms may lie free in the sub cutis. Intact worms and the

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microfilariae in the skin, eye and other organs do not elicit immune reaction. As the microfilaria aged and dye they invoke immune responses (McKenzie *et al*., 1992). Direct parasite attrition is not known to play any part in the development of various skin and ocular lesions attributed the disease process. It has been strongly advanced that the multiplicity of immune responses (both cellular and humoral) particularly antibody reactions could play a role in antibody dependent cell-mediated microfilaria clearance and immunopathology (Titanji *et al*., 1992). Like any other parasite, specific antibodies in onchocerciasis are studied mainly to understand their serodiagnostic function (Boatin *et al*., 1998; Vincent *et al*., 2000), their probable involvement in immunopathogenesis and in understanding the mechanism of immunity needed for vaccine development (Green *et al*., 1985). Antibody assay could also be of value in drug screening and monitoring of control programme (More and Copeman 1992). There are several reports on studies to measure both polyclonal and parasite-specific antibodies in onchocerciasis patients. Such studies were aimed at determining the role antibodies responses play in the pathogenesis of onchocerciasis (Engelbrecht *et al*., 1992). With the absence of a suitable animal model for the study of onchocerciasis, this type of investigation is carried out by measuring circulating antibodies in clinically defined onchocerciasis patients with or without obverts signs of the disease. Hence, this present paper report on study aimed at establishing if the recombinant antigen, Ov1.9 can be a useful serodiagnostic marker. Secondly, establish if there is any relationship between serum antibody responses to Ov1.9 and disease clinical manifestations. Thirdly, investigate if the earlier impact of initial dose of ivermectin treatment on serum antibody levels may be used to monitor treatment and drug screening. Lastly, investigate its suitability as tool for surveillance for recrudescence which inevitable is the cornerstone of any disease control and elimination programmes (Guzman *et al*., 2005). On-going ivermectin control will depend on a simple and sensitive test that can easily be applied under field condition.

MATERIALS AND METHODS

Sample Population

The sample population has been described previously (Osue *et al*., 2008; Osue *et al*., 2009). The cases (n=95) are categorized according to clinical and parasitological infections. Briefly, 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 parasitological and 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 hours 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.

Recombinant Antigen

A recombinant antigen coded Ov1.9 was a Glutathione S-transferase (GST) fusion protein derived from the *in vitro* translation of mRNA from *O. volvulus* adult worm genomic and cDNA clone isolated from a lambda gt11 vector expression library. Briefly, intact female adult worms of *O. volvulus* were recovered from excised nodules by enzyme digestion. The nodules were incubated in 0.3% (w/v) collagenase in RPMI 1640 medium as described (Engelbrecht and Schulz-Key, 1984). Isolation of RNA for the molecular cloning of *O. volvulus* was carried out in hot phenol/SDS as described by Taylor *et al*., (1984). The OV1.9 antigen was screened from the translation products using reactivity with onchocerciasis human infection sera. The mRNA, genomic DNA and protein sequence identity of this antigen is presently not available from *O. volvulus* cDNA expression library database. The hyper-immune serum raised against the antigen showed it is associated with structures of *O. volvulus* microfilaria and adult worm cuticle. The antigen was prepared and supplied to the Immunology Research Laboratory, N.I.T.R., Kaduna, by Dr. G. Braun (Cambridge University, London).

Indirect Sandwich ELISA

Serum IgG antibody was measured using a modified standard protocol (Engelbrecht *et al*., 1990). Briefly, the procedure and concentration of reagents and buffers are as follows: wells of microtitre plates were sensitized with antigens diluted in carbonate/bicarbonate buffer (pH 9.6) at optimum concentration, 150µl per well and incubated overnight at 4^0C. All other steps were performed at room temperature (RT^o) with a minimum of 5 washings in between. The unspecific sites were “blocked” with 1-2% bovine serum albumin (BSA) at 200µl per well for 1hr before serum was added at 100µl per well for 2hrs. Anti-human IgG horseradish peroxides conjugate was added for IgG assay at 150µl per well for 1hr 30min. Antigen and antibody reactions were detected by addition of substrate solution made up of orthophenylene diamine

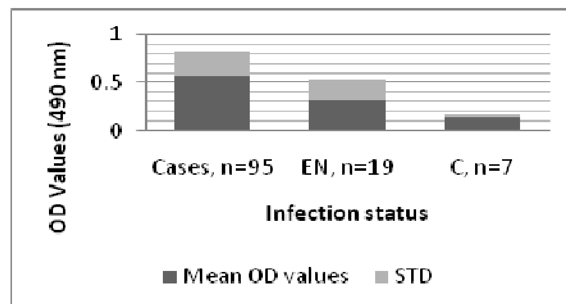

Figure 1 IgG reactivity to *O. volvulus* recombinant Ov1.9 antigen. Non-endemic controls (C), endemic normals (EN) and sample size (n). Antibody levels are given as mean optical density (OD) at 490 nanometer (nm) wavelengths.

(OPD) with hydrogen peroxide (0.02M) in citric acid (0.1M); Na_2HPO_4 buffer at 150µl per well and allowed to stay for 15minutes. The reaction was terminated with 2M H_2SO_4 at 30µl per well and rock gently for 5min. Quantization of the antigen-antibody reactivity were done by measuring the optical density (OD) of wells of microtitre plates in a Dynatech ELISA reader (model MR4000) at 490nm test filter and 650nm reference filter.

Positive and negative reactions were determined from cut-off point of mean OD values plus (+) two (2) standard deviation (SD) of non-infected normal controls (n=7). Correlation between the detection of antibodies and level of skin microfilarial load or clinical signs (those with acute or chronic skin and optic nerve diseases etc.) were sought. The sensitivity and specificity of assay were calculated based on percentage positives in confirmed onchocerciasis patients and in control. Overall, the IgG antibody level was higher in proven clinical and parasitological cases (n=95) with mean optical density (OD) values of 0.58± versus 0.37±. The non-endemic (EN, n=7) control showed a slight background reaction with a mean OD values 0.12±0.02. The difference was statistically significant (P<0.05) by t-test of unpaired data.

RESULTS

Antibody Responses to Ov1.9

Indirect sandwich enzyme-linked immunosorbent assay (ELISA) was used to measure serum IgG antibody responses to a recombinant antigen of *Onchocercal volvulus* designated (Ov1.9). The antigen was reacted with sera obtained from residents (n=114) of onchocerciasis endemic area prior to commencement of ivermectin treatment. They comprised those with clinical and parasitological evidence of infection (n=95) and from the assumed non-endemic (NE) individuals (n=19) without any evidence of infection and with paired sera (n=32) obtained 2-months post-treatment. The IgG antibody level was higher in proven clinical and parasitological cases (n=95) with mean optical density (OD) values of 0.58± versus 0.37±. The non-endemic (EN, n=7) control showed a slight background reaction with a mean OD values 0.12±0.02 (Fig.1). The difference was statistically significant (P<0.05) by t-test of unpaired data.

Antibody Responses by Gender and Age

Table 1 shows there was no difference between the male (n=42) and female (n=53) with mean OD values of 0.50±0.23 and 0.53±0.25, respectively. Similarly, there was no difference between the younger age sub-group (15-29yrs, n=24) and the older age sub-group (≥50 yrs, n=30) the two sub-groups were higher than the middle age sub-group (30-49yrs, n=41). The observed difference was not statistically significant by T-test of unpaired data (P>0.05).

Analysis of Antibody Levels According to Infection Status

Figure 1 showed there were differences between the sub-group with evidence of clinical sign but were both nodule and microfilaria negative (N-mf-) and those with or without palpable nodule and/or skin microfilaria (N-mf+, N+mf-, N+mf+). Among the infection group, the sensitivity of the assay showed 87.3% (83/95), 88.7%, (55/62) in skin mf positive, 87.9% (28/33) for those without skin mf and 63.2% (12/19) among the EN.

Analysis of Antibody Levels Based on Clinical Signs

Antibody levels in clinically defined sub-groups (Fig. 2) showed that those with optic nerve disease (OND, n=12) had the least mean OD values compared to those without any visible clinical manifestations of the disease (CS-ve, n=39) followed by those with papular onchodermatitis (POD, n=18) and those with chronic skin disease (CSD, n=20) had the highest OD values. The difference

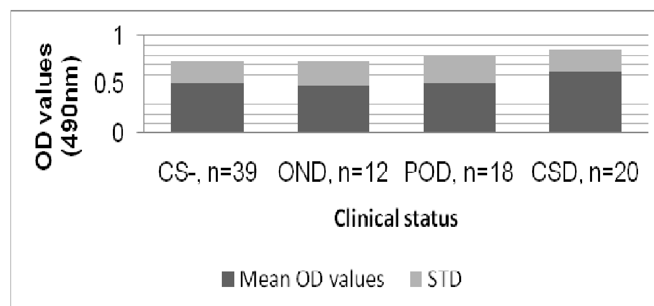

Figure 2 IgG antibody responses to Ov1.9 in clinically defined groups of patients. Clinical sign negative (CS-), optic nerve disease (OND), papular onchodermatitis (POD), chronic skin diseases (CSD) and sample size (n).

between CSD and other sub-groups was statistically significant by T-test of unpaired data (P<0.05).

Effect of Ivermectin on Antibody Responses to Ov1.9

The antibody levels of patients sera (n=32) randomly selected from the initial sample population (n=114) obtained two months after ivermectin treatment were tested and compared with their pre-treatment values. There was no significant difference between the mean OD values of the pre- and post-treatment groups (data not shown). Only 8/32 (25%) of paired post-treatment sera had increased antibody of 10% and above.

DISCUSSION

Serum antibody reactivity to Ov1.9 recombinant antigen revealed a diverse response pattern in patients with infection and overt disease. The observed difference between infected and non-infected EN may be a characteristic of its serodiagnostic potential as a reagent for screening test. The sensitivity was not high enough compared to those reported by others (Weil *et al*., 2000, Andrew *et al*., 2005). The finding showed that IgG antibody response to Ov1.9 showed strong association with chronic skin disease and possibly contribute to underlying pathology.

The Ov1.9 has been reported to be localized on surface coat of microfilaria and adult worm (Braun *et al*., 1991). The antibody level did not show any correlation with infection status as shown on Table 1. Observed difference between the cases and endemic normal or non-endemic control was statistically significant by t-test of unpaired data (P<0.05). Yet, with the very low sensitivity and specificity rates recorded, the antigen proved not suitable as a diagnostic reagent. The serodiagnostic reliability may be improved upon if made a component of cocktail recombinant antigen as widely reported by others (Chandrashekar *et al.,* 1996, Bradley *et al*., 1998, Rodriguez-Perez *et al.,* 2002). The need for an additional IgG4 assay step to enhance specificity by Weil *et al*., 1990) has been countered by Bradley *et al*., (1993) observation that antibody response to low molecular weight (LMW) antigens is IgG subclass restricted.

The heightened IgG antibody responses to OV1.9 in those having CSD compared to those with OND as shown on Fig. 2 was significant by t-test of unpaired data (P<0.05). This outcome tallies with earlier reports by Engelbrecht *et al*., (1991), Mudoch *et al*., (1996) and Osue *et al*., (2008). It has further re-enforced the positive correlation of parasite-specific antibodies with the development skin manifestations. Conversely, the low level of OV1.9 antibody responses in OND subgroup is indicative that circulating parasite-specific antibodies may not be involved in the pathology and pathogenesis of ocular lesions. Various studies have demonstrated that autoimmune response elicited by cross-reactivity between host and parasite (Braun *et al*., 1991; McKechnie *et al*., 1993a; 1993b) with retinal pigment epithelium (RPE) antigens has been documented. In addition, immunological reaction to dead microfilariae elicited in the eye has been implicated in the pathogenesis of ocular lesions. This involves antibody-dependent cell-mediated cytotoxicity (ADCC) involving human eosinophils, neutrophils, macrophages and platelets. The chances of developing CSD are more plausible in those with higher parasite-specific antibody levels. More or so, no mechanical or direct parasite attrition has been implicated in the disease clinical manifestations.

The lack of suitable animal model has made measuring changes in circulating antigen levels or antibody response to parasite-specific antigens after treatment of patients the best option for assessing efficacy of treatment control strategy. From this study, the absence of remarkable change in antibody response to Ov1.9 two months after treatment showed that it will not be suitable for use as reagent for monitoring drug screening against the *O. volvulus* as suggested by More and Copeman *et al*., (1991). Alternatively, naturally infected cattle has been adapted as a model for screening microfilaricidal or

macrofilaricidal compounds (Gilbert *et al*., 2005). A single antigenic epitope with high diagnostic reliability, easily released and detectable or stimulate increase level of antibody responses after treatment will be ideal. Scarcity of *O. volvulus* parasite materials has been addressed with recombinant DNA providing reliable source of obtaining antigen. With only few individuals (25%) having ≥ 10% increased antibody is an indication of possible selective immunotolerance to secondary or anamnestic response by others with no response. A probable clinical implication of this poor or non-response is seen after administration of purified protein derivatives (PPD) and tuberculin skin tests with bacillus Calmette-Guérin (BCG) vaccination (Rougemount *et al*., 1977; Elkhalifa *et al*., 1991). The immunodepression among onchocerciasis patients these products have not been reproduced.

We conclude that antibody responses to recombinant Ov1.9 antigen support the involvement of immunopathology in the etiology of chronic skin clinical outcome. This study has proved the role parasite-specific antibodies played in the pathogenesis of chronic onchodermatitis and conversely eye lesions are not circulating antibody-dependent. The importance of this investigation is to identify candidate antigens that could be used as vaccine to prevent infection or development of clinical diseases.

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