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Schistosome Specific Antibodies in Individuals Co-Infected with Malaria in Southwest Nigeria

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Abstract

A cross-sectional study design in two primary schools in Ibadan and Akure was used to determine the prevalence of urinary schistosomiasis, and the human humoral immune response to schistosome antigens in individuals with malaria co-infection. Urine samples were collected from 163 children, while 112 gave blood samples. Malaria parasitaemia was determined by microscopy after Giemsa staining and schistosomiasis by centrifugation technique. Serum samples were analyzed for antibodies to crude *S. mansoni* soluble egg, adult worm antigens, and crude *S. haematobium* egg antigen by ELISA. The sample population consisted of 40% (62/163) infected with schistosomiasis, 31% (50/163) with malaria, and 6% (10/163) co-infected. All the co-infected students had asymptomatic malaria with parasite densities ranging from 200 - 4,420 parasites/ul blood. IgG titres to the various *Schistosoma* antigens did not vary significantly. However, antibody titres to the soluble egg antigen increased with age of volunteers. Antigen specific isotype distribution showed a higher prevalence of IgG3 and IgG4.

Keywords: Schistosomiasis, antibodies, co-infection, malaria, ELISA

Introduction

Malaria and helminth infections are the major parasitic diseases in developing countries and their epidemiological coexistence is frequently observed, particularly in Africa [1, 2]. Co-infection with *Plasmodium falciparum* and *Schistosoma* species, especially *Schistosoma haematobium* may have an important influence on the regulation of inflammatory factors associated with the development of these infections and their respective morbidity [1]. The implications of concomitant malaria and helminth infections in a co-infected host have been explored in animals under laboratory conditions, and a growing number of studies in the human population have been conducted with contradictory results [reviewed in 3].

Currently, an estimated 200 million people in over 74 countries have schistosomiasis; 120 million of them have symptoms, and 20 million have severe illness [4]. Among parasitic diseases, schistosomiasis is believed to rank second behind malaria in global importance. *S. haematobium* is the predominant species in Africa, and urinary schistosomiasis caused by *S. haematobium* is observed to be more prevalent in Nigeria than intestinal schistosomiasis because of the wider distribution of its snail host *Bulinus* species [5], alongside indiscriminate urination of eggs into water bodies containing the snail host [6]. Possible consequences of *S. haematobium* infection include haematuria, dysuria, nutritional deficiencies, lesions of the bladder, kidney failure, and an elevated risk of bladder cancer and in children, growth retardation. Accordingly the estimates for morbidity and mortality in affected populations are high [7]. Schistosome infections are common wherever climatic and environmental conditions favour snail development and where social and socio-economic conditions allow faecal and urinary contamination of water that is used for drinking, bathing, washing or working while it correlates with the breeding season of mosquitoes which causes intense transmission of the malaria parasite.

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The prevalence and intensity of urinary schistosomiasis in endemic areas show an infection pattern which seems to peak in individuals in the first two decades of life, with varying rates [8, 9]. When compared with infected adults, children with schistosomiasis notably harbour a greater worm burden and are more intensely infected due to the high parasite transmission rates and the frequency of exposure to infection sites [8]. Individuals who are encountering the infection for the first time however, may have severe infections irrespective of their age and sex [6]. School-age children usually present with the highest prevalence and intensity of *S. haematobium* infection [10-12], and re-infection following parasitological cure [13]. However, negative health consequences are not limited to this group since high intensity infections can cause serious chronic disease long after initial infection.

Immune responses to schistosomiasis can be summarized as either anti-egg or anti-worm. Immune responses against antigens that are secreted by the egg stage include granulomatous hypersensitivity, antibody production, anti-fecundity, and transmission blocking immunity, some of which are characterized as protective and mediate resistance to reinfection [14-16]. In many people from endemic areas, treatment with praziquantel (PZQ), increases parasite-specific immunoglobulin E (IgE) and other Th2 responses in the months following therapy, responses that have been associated with subsequent resistance to re-infection [17]. In young children and adolescents, re-infection is common requiring frequent treatments with the potential to promote drug resistance and often leading to severe clinical consequences [18]. Elevated IgG1, IgA and IgE in urinary schistosomiasis are associated with Th2 responses and levels of different classes of immunoglobulins vary at different stages of urinary schistosomiasis [18, 19]. It has been suggested that there is need for further studies on humoral and cellular responses to schistosomiasis and the co-incidence of other infections, since polyparasitism is common among populations in developing countries and there is limited information on interactions between these parasites in this part of the world [20].

This study was conducted to assess the prevalence of schistosomiasis and the immune responses *to S. mansoni* and *S. haematobium* antigens in school aged children who may be co-infected with malaria in Odo-ona, an endemic area in Ibadan, Oyo state and Ipogun village, an endemic area in Ifedore Local Government Area, Ondo.

Methodology

Study Areas

The study was carried out in two different locations; Odo-Ona (7°24 N; 3°48 E), Ibadan, Oyo State and Ipogun ((7°19` N; 5°05`E), Ondo State both located in the south-western part of Nigeria. Previous studies [11, 21] indicated schools in Ibadan and Ifedore Local Government Area, Ondo state, where schistosomiasis is endemic. One of such schools was chosen in Ibadan, and two were chosen in Ifedore Local Government Area. The schools selected were Olubi Memorial Grammar School, Kudeti, Ibadan and Morohunkeji Nursery and Primary School, Ipogun in Ifedore Local Government Area, Ondo state. School pupils were examined for prevalence of schistosomiasis and malaria, and the humoral response to schistosome antigens among individuals with schistosomiasis and malaria co-infection.

Study design

A cross sectional study design was used for this survey. Urine samples were collected from a total of 163 school children between 1000hrs and 1300hrs. The final sample size for each school was influenced by the degree of co-operation of both the pupils and their parents. Some of the pupils' parents did not consent for their children to participate in the study.

Urine (10mls) was collected from each student and examined for *S. haematobium* eggs using the centrifugation method as described by Piekarski *et al.* [22]; the frequency of visible haematuria (macrohaematuria and microhaematuria) was noted and recorded. Each student provided information on his/her area of residence, water contact activity and water usage. Single urine and blood samples were used to determine parasitic loads (malaria parasite density and *S. haematobium* egg load. Blood samples (3ml) were collected intravenously from each school child by trained medical personnel for assays of the humoral immune responses to the antigens in the laboratory. Schistosomiasis infected participants were treated with Praziquantel (Biomedecine S.P.R.L, Belgium) at 40mg/kg body weight. Samples were collected at the Ibadan site in

August, 2006 while samples were collected at the Ipogun site in December of the same year.

Ethical Considerations

Recruitment of children was done through the parent/guardian of each child by assenting to the informed consent form. Ethical approval for the study was obtained from the joint U.I/UCH ethical review board, Ibadan. Results were treated as confidential.

Antigens

Schistosoma mansoni soluble egg antigen (**SEA**) 3mg/ml and soluble adult worm antigen (**SWAP**) 3mg/ml were obtained from the Schistosome Biologicals Supply Company (Egypt). Crude *S. haematobium* egg antigen was also used.

Blood

Blood (3ml) was collected into ethylene-diamine-tetraacetic acid (EDTA) anticoagulant. The blood samples were centrifuged in the laboratory at 9,000 revolutions per minute (r.p.m) for three minutes; plasma samples recovered were stored at -80°C.

Parasitaemia

Thick films were made and stained with 1% Giemsa stain for thirty minutes, washed off and allowed to air dry. The slides were examined under the x100 objective lens. Parasitaemia was determined by counting 200 white blood cells (WBC) on every field and then counting the number of malaria parasites on the same field three times.

Total IgG Antibody Responses to Schistosome Antigens

The humoral response to schistosome antigens was determined by Enzyme Linked Immunosorbent Assay (ELISA). 96 well microtitre plates were coated with 100µl each of *Schistosoma mansoni* Soluble Egg Antigen (SEA), Soluble Adult Worm Antigen (SWAP) and crude extract of *Schistosoma haematobium* egg antigen at 1:500dilution in carbonate buffer (pH 9.6) and incubated overnight at 4C. Excess buffer was poured away and the wells blocked with 200µl of blocking buffer and then incubated for 1 hour at 37C. Plates were washed three times with PBS/0.05%Tween 20. Serum samples diluted 1:200 in blocking buffer were added at 100µl/well to each well and the plate incubated at 37°C for 1 hr. After three washes, horseradish peroxidase conjugate (IgG/HRP) antihuman IgG at a dilution of 1:2000 in blocking buffer was added to each well and the plates incubated for 1 hour at 37C. Plates were washed three times with PBS/0.05%Tween 20 washing buffer. The enzyme reaction was developed by the addition of 1:1 peroxidase solutions as substrate {(hydrogen peroxide (H_2O_2) and ABTS {2,2-Azino-bis (3- ethyl Benzthiazoline)} and incubated for 30 minutes at 37C. The reaction was stopped with 10% SDS. Plates were read at an optical density of 405nm using an ELISA plate reader (Molecular Devices, CA).

Anti - SEA Specific IgG Subclass ELISA

96 well microtitre plates were coated overnight at 4°C with SEA at a 1:500 dilution in carbonate buffer (pH 9.6). Excess buffer was poured off and plates blocked with 1% BSA Tween 20 and incubated for 1 hour at 37°C. Plates were washed thrice with PBS/0.05% Tween 20. After three washes, 1:200 dilution of serum were made in blocking buffer and added at 100µl/well to each of the well and the whole incubated for 1 hour at 37°C. Plates were washed thrice with PBS/0.05% Tween 20 and monoclonal antibodies to IgG1, IgG2, IgG3 and IgG4 were added at 1:400 dilution, and the whole incubated for 1 hour at 37°C. Plates were washed thrice with PBS/0.05% Tween 20, mouse antihuman IgG conjugated to horseradish peroxidase diluted 1:2000 in blocking buffer was added and the plates incubated for 1 hour at 37C. Plates were washed three times with PBS/0.05%Tween 20 washing buffer. Enzyme reaction was developed by the addition of 1:1 peroxidase solutions as substrate (hydrogen peroxide (H_2O_2) and ABTS) and incubated for 30 minutes at 37C. The reaction was stopped with 10% SDS and plates were read at an optical density of 405nm using the ELISA plate reader, (Molecular Devices, CA, USA).

Data Analysis

Data analysis was done using Microsoft 2000 Excel and Prism Graphpad statistical package. Correlations were analyzed with spearman's correlation co-efficient. Mean values were plotted as bar chart and scatter plots were used to illustrate the observed correlations.

Results

Urine examination

All the urine samples from the 50 volunteers in Ibadan were negative for schistosomiasis by microscopy, although three of the students claimed that they had seen blood in their urine samples before and had been treated. In Ipogun, 113 volunteers participated (Table 1), and urine samples from 11 children had visible haematuria (macrohaematuria) while samples from nine others, only showed visible haematuria (microhaematuria) after centrifugation. The egg output in the infected children in this study was highest in the 8-10 years age group. The prevalence of schistosomiasis infection in Ibadan was 0%, and 55% in Ipogun, where 33 boys (53%) and 29 girls (47%) were infected (Table 1). Of the 29 girls that were positive for schistosomiasis, 15 (52%) had high parasitaemia (=50 eggs/10ml urine). The highest intensity of infection in the study was observed in a 11 year old boy with body weight of 29kg (120 eggs per 10ml of urine), while lowest intensities were observed in two children, a 10 year old boy with body weight of 29kg and a 8 year old boy with body weight of 25kg (1 egg per 10ml of urine).

Table1: Prevalence of urinary schistosomiasis in the study sites Ibadan and Ipogun

	Ibadan		Ipogun	
	No examined	No infected (%)	No examined	No infected (%)
Male	31	0 (0)	59	33 (53)
Female	19	0 (0)	54	29 (47)
Total	50	0 (0)	113	62 (54.87)

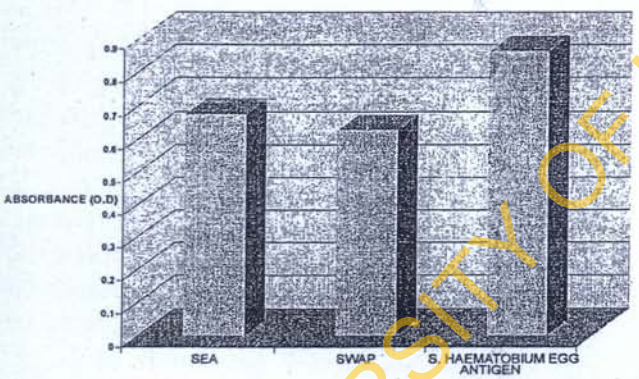

Fig 1: Antibody response to the three tested antigens among the sample. The highest antibody response was produced to *S.haematobium* egg antigen

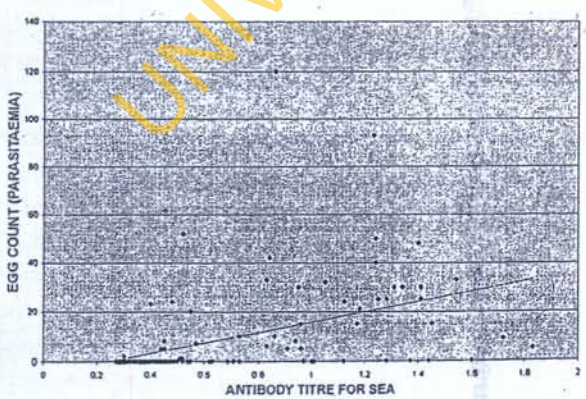

Fig 2: Correlation of egg count and antibodies to *S. mansoni* soluble egg antigen

Malaria microscopy

The malaria parasite rate in Ibadan was 54%, with 27/50 pupils having parasite densities ranging from 200 to 4,420 parasites/uL of blood. In Ipogun, malaria parasite rate was lower at 37%, with 23/62 pupils having parasite densities of between 200 to 720parasites /uL of blood. All the malaria positive pupils were not symptomatic. Ten pupils only were found with malaria and schistosomiasis co-infection.

Antibody Response to Schistosome Antigens within the Population

The children in the study population produced high levels of anti-schistosome antibodies as measured by ELISA to three schistosome antigens. Antibody response increased with increasing parasitaemia (Fig 2). Among the positive individuals only, the same trend was observed (r= .3853; 95% C.I =. 07429-.6280). Increase in antibody titre correlated with increase in age among the positive individuals (r=.3320; 95% C.I=.01404-.5900).

Comparison of Antibody Response to Each of the Three Tested Antigens

The study revealed that all the children produced high antibody responses to *S. haematobium* crude antigen followed by *S. mansoni* soluble egg antigen (SEA) and then adult worm antigen (SWAP) (Fig.1). However, among individuals positive for schistosomiasis only, the highest response was produced against the crude *S. mansoni* egg and crude *S. haematobium* antigen than to *S. mansoni* worm antigen.

IgG Subclass/Isotype Distribution of S. Mansoni Soluble Egg Antigen within the Study Population

Serum samples (112) were analysed for IgG subclass antibody to *S.mansoni* soluble egg antigen (SEA). IgG3 isotype predominated across the

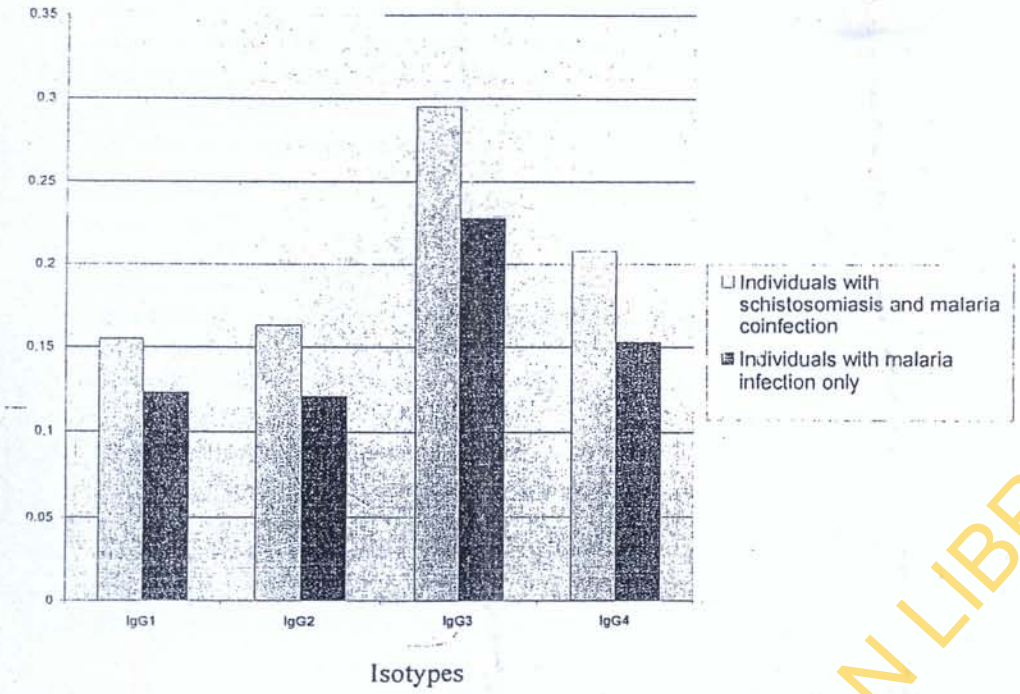

Fig. 3: IgG isotypes in individuals with schistosomiasis and malaria co-infection and those with malaria infection only.

entire population, followed by IgG4, IgG2 and IgG1. IgG subclass levels were observed in the following order IgG3>IgG4> IgG2 > IgG1 among individuals with schistosomiasis infection alone. IgG3 and IgG4 predominated among individuals who were negative for schistosomiasis but positive for malaria. In 10 individuals with schistosomiasis and malaria co-infection, it was observed that IgG3 and IgG4 also predominated over IgG1 and IgG2 (Fig 3). In comparing *Schistosoma* specific IgG isotype distribution between individuals with single infection (malaria) and those with co-infection (schistosomiasis and malaria), it was observed that IgG3, IgG4, IgG2 and IgG1 were higher in individuals with co-infection compared to those with single infection (Fig 3).

Discussion and Conclusion

This study was designed to determine the prevalence of schistosomiasis amongst schoolchildren in Ibadan and Ipogun, and to determine antibody responses to schistosome antigens among children with malaria co-infection. The study showed that *S. haematobium* is endemic in Ipogun, Ondo State, which is a rural area in Nigeria, as earlier reported [12, 14].

The prevalence of schistosomiasis infection (55%) observed in Ipogun in this study may indicate increasing use and contamination of natural water sources by the growing population in Ipogun as well as poverty, as observed in the lack of good sanitary facilities and lack of pipe-borne water which could prevent human contact with these contaminated water sources. The higher prevalence rate observed in males (53%) in this study conforms to patterns found in most endemic areas [11, 13] and reflects the greater opportunities that males have for exposure to the infection; it also establishes the fact that boys generally are more engaged in water contact activities such as swimming, playing and bathing in contaminated rivers or streams than girls, probably because they have more time to play.

This study also revealed a probable cessation in transmission of schistosomiasis at the Ibadan study site from 11.6% in 1999 [11,] to 0% in 2007. Evidently, reduced contact with water bodies through the behavioural profiles of the students (older children 14-16 years tended not to have water contact) and the construction of bridges over the water on the way to the school in the study area, may be responsible for the cessation in transmission of the disease. Factors which influence the transmission of schistosomiasis in an endemic area include the presence of snail intermediate hosts of the parasites and most importantly, human-water activities [11, 12, 21].

Elevated levels of IgG3 and IgG4 were observed generally across the entire studied population while IgG1 and IgG2 had similar levels, though lower than that observed in IgG3 and IgG4. In individuals with schistosomiasis that were negative for malaria, IgG3levels were highest and IgG1 levels were lowest. Elevated IgG3 observed in this study in these individuals agrees with previous work. Low IgG1 levels observed in these individuals agrees with other data [22] showing that IgG1 responses to soluble egg antigens usually decrease with age, independent of intensity of infection. High IgG4 levels observed seem to indicate intense infection [12, 19, supporting the idea that IgG4 might block a protective role of IgE in most infected individuals [25]. Certain antibodies present in the sera of chronically infected subjects compete with IgE antigen binding, and IgG4 accounts for most of this blocking activity. Immunoglobulin isotypes IgE, IgG4, and IgG2 have significant roles in the human response to *S. mansoni* infection [30].

In individuals that were positive for malaria but negative for schistosomiasis, IgG3 antibody levels were found to be the highest. IgG3 responses are associated with fewer fever episodes as well as less anaemia [25]. However, since no malaria antigen was tested in this study and IgG3 was observed to be high among these individuals, it may be suggested that there are cross reactivity responses between the *S. mansoni* antigens and *P. falciparum*. Some Brazilian subjects who were not exposed to malaria had some anti-*Plasmodium* IgG3 activity, suggesting that cross reactive schistosomal antigens may also be capable of inducing IgG3 isotype antibodies although not as effectively as in malaria infections [25].

In the ten individuals that had malaria and schistosomiasis co-infection, IgG3 and IgG4 predominated over IgG1 and IgG2. Children with schistosomiasis who were also infected with malaria parasites had higher levels of schistosome specific IgG3 than children who were free of malaria [23]. High anti-SEA IgG3 was observed in Kenyan school children who had malaria and schistosomiasis infections suggesting that this was driven by cross-reactive *P. falciparum* epitopes [24]. Though all the schistosome- specific IgG isotypes were observed in all the participants, those with co-infection had significantly higher IgG isotype titres than those with single infection. This could be because malaria infection generally favours the production of IgG3 since children will be exposed to malaria earlier in life than to schistosomiasis. IgG3 is a dominant isotype in malaria-only infections but not in schistosomiasis-only infections [23]. As has been suggested previously [25], both parasites could possess molecules that bind human IgG3 via an isotype-specific component of this antibody. The reason why this cross-reactivity predominantly appears to be restricted to the IgG3 isotype remains an interesting question.

Conclusion

It is believed that the mechanisms by which interactions occur between parasites is still limited and a protection-pathology balance is at the heart of understanding of immunity to malaria. There is need for further studies on the implications of this co-infection as well as immunological interactions that occur between both parasites. It is important to understand that solutions to these tropical diseases depend more on an all-round control programme which will not eradicate one to the favour of another but will instead, take care of diseases that are co-endemic in individuals.

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