

Full Length Research Paper

IgG Enzyme-Linked Immunosorbent Assay (ELISA) for immunodiagnosis of *Schistosoma haematobium* infected subjects living in an endemic Nigerian village

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***Schistosoma haematobium* infects school children in areas where the disease is endemic. This study was carried out to determine the reliability of IgG Enzyme-Linked Immunosorbent Assay (ELISA) as an immunodiagnostic tool, using whole antigen derived from *Schistosoma* eggs. Children of school going age living in Ipogun village, in south western Nigeria; a schistosomiasis endemic community; were used in this study. The sensitivity of the IgG ELISA in detecting positive cases was 68.0% and there was a significant correlation ($P < 0.05$) between IgG titres and the intensity of infection. However, the specificity was 59.3%, slightly lower than the sensitivity. Results from this study shows that IgG ELISA can be used as a diagnostic tool for determining *S. haematobium* infection, as it provides an evidence for the intensity of the infection in the infected individuals.**

Key words: Urinary schistosomiasis, enzyme-linked immunosorbent assay, IgG, intensity, immunodiagnosis.

INTRODUCTION

Schistosomiasis, also known as bilharziasis, is a parasitic disease that leads to chronic ill health. The disease is indicated either by the presence of blood in the urine or, in the case of intestinal schistosomiasis, by initially atypical symptoms which can lead to serious complications involving the liver and spleen (WHO, 2004). Schistosomiasis affects 200 million people across the globe and is directly responsible for an annual death of 20,000 patients (Barsoum, 2003). It is second to malaria in the world's parasitic diseases in terms of endemicity and the number of infected people (Utzinger et al., 2001). Three major species are responsible for infection in humans: *Schistosoma haematobium* in Africa, *Schistosoma mansoni* in Africa and South America, and *Schistosoma japonicum* in the far east (Barsoum, 2003).

Unfortunately, schistosomiasis is a disease that primarily results from lack of education and public health

facilities, appalling sanitary conditions, and poverty found in many underdeveloped nations in Africa. Increasing population density in Africa has put heavy pressure on effective land usage, leading to a progressively widespread development of large- and small- scale irrigation schemes that increase the potential for transmission (Butterworth, 1990). Human exposure to freshwater in underdeveloped tropical and sub-tropical areas suffering from these problems is the major determinant to infection. In Africa, endemicity may be linked to behaviour and the socio-economic status of the people living in this part of the world (Ukoli, 1987,1991, 1992). The factors that promote transmission include the presence of susceptible snail intermediate hosts in stagnant or freshwater bodies visited by people living around such for domestic, occupational, or recreational activities. In Nigeria, two species of the causative organisms have been extensively reported. These are *S. mansoni* and *S. haematobium*, causing intestinal and urinary schistosomiasis respectively; with the latter being more widely spread (Ejezie et al., 1989). Although, most

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infections occur in residents of endemic areas, it has been clearly documented that brief exposure to freshwater is sufficient to establish infection; thus, travellers may also be infected.

Diagnosis of infection is crucial to disease management. *S. haematobium* is diagnosed by the presence of ova in urine or tissue. However, ova loads are not always sufficient for diagnosis, especially in long-standing chronic illness and cases of light infections (Goncalves et al., 2006). Other diagnostic tools used include ultrasonography and echodoppler cardiography (Lambertucci et al., 2000); immunofluorescent antibody tests and antigen detection assays are also increasingly used. Enzyme-Linked Immunosorbent Assay (ELISA) has been reported to be an effective immunodiagnostic tool (Turner et al., 2004; Mutapi et al., 2006) and its relevance in disease diagnosis is increasing. The sensitivity and specificity of ELISA in schistosomiasis diagnosis has been documented, studies have shown different reports of the reliability of the egg-antigen ELISA based immunodiagnosis. Lin et al. (2008) reported a sensitivity of 73.3 to 87.4%, with a lower specificity value of between 38.9 and 53.5%. Goncalves et al. (2006) suggested a screening method that combines antibody isotype detection and repeated parasitological stool examination in the bid to increase the chances of detecting *S. mansoni* in infected patients. The latter's recommendation was based on their findings following an immunodiagnostic procedure. This study evaluates the immunodiagnostic precision of IgG by ELISA in detecting schistosomiasis infected children in an endemic village in Southwest Nigeria.

MATERIALS AND METHODS

Study site

The study was carried out at Ipogun village (7°19' N; 5°05' E), a rural community in Ifedore Local Government Area of Ondo state, south west Nigeria. The village had an estimated population of about 6,000, and is about 14 km away from Akure, the state capital. The major occupation of the villagers was farming.

Compliance with study protocol/ethical considerations

Ethical clearance was provided by the Ondo State Ministry of Health. Written informed consent was sought from the parents and guardians of the children before the study began. Results were made known to the parents and all infected children were treated with praziquantel

Urine collection/analysis

Urine samples were collected between 09:00 and 13:00 h in transparent 20 ml plastic bottles. The bottles were labelled, and taken to the laboratory for analysis. Laboratory analysis was done using the centrifugation method (Chugh et al., 1986). 10 ml of urine was centrifuged at 1,500 rpm for 3 min and the residue examined under a X10 objective of a microscope for the presence of terminal

spined ova of *S. haematobium* which were expressed as the number of *S. haematobium* eggs/10 ml urine.

Blood collection

Based on the results of the urine analysis, 3-5 mls of blood was collected by venipuncture from 173 students in three schools by qualified health workers. The blood samples were collected from the following category of subjects:

1. Subjects who were positive for *S. haematobium* (87) following urinalysis in Ipogun
2. Subjects who were negative for *S. haematobium* (61) following urinalysis in Ipogun
3. Subjects who were negative for *S. haematobium* (25) following urinalysis but living in Akure, the capital city (control).

The blood was collected in sample tubes containing EDTA and transferred to the laboratory in picnic boxes filled with ice; the samples were centrifuged at 4000 rpm for 3 min. The supernatant (plasma) was pipetted into vials and frozen at -80°C until analysed.

Antigen processing

Crude egg antigen was prepared by homogenising the schistosome eggs recovered from urine samples of infected subjects. The eggs were washed three times in Phosphate Buffered Saline (PBS) by centrifugation before crushing in mortar with a pestle. The crushed homogenate was also centrifuged and the supernatant was then stored in a vial at -80°C until used.

Laboratory procedure

IgG ELISA (Pardo et al., 2004) was used to determine the *S. haematobium* positive subjects. Standardization of the reagents, antigen and serum was done to determine the optimum working dilutions. Polystyrene microtiter plates (Nunclon, Nalge Nunc International, Denmark) were coated with 50 µl per well of crude egg antigen diluted 1:200 in carbonate buffer (pH 9.6), and then incubated overnight at 4°C. The plates were washed three times with 0.05% Tween 20 in PBS-Tween 20. Washed plates were dried on blotting paper and 150 µl of blocking buffer; 1% Bovine Serum Albumin in Phosphate Buffered Saline (1% BSA/PBS); was introduced and the plates were incubated for 1 h at 37°C. The plates were washed again three times with PBS-Tween 20. 50 µl of serum samples was added to the plate in duplicates at a dilution of 1:100 in 1% BSA in PBS, and incubated for 1 h at 37°C. The plates were washed three times with PBS-Tween 20. 50 µl of Horseradish Peroxidase anti-human immunoglobulin G conjugate (Sigma, St. Louis, MO, USA) was added per well at a dilution of 1:2000 and the plate was incubated for 1 h at 37°C. The plates were washed with PBS-Tween 20, and 50 µl of substrate solution; 2,2'-azino-di-3-ethyl-benzthiazoline sulfonate [ABTS] was added. The reaction was allowed to proceed in the dark for 30 min at 37°C and the absorbance read at 620 nm with a micro plate reader (Biotrak II Reader). The assays included a reference high positive sample (SUB ID 6) and negative controls (SUB IDs 236 and 237). The cut off (0.086) was calculated as $2[SD + \bar{X}]$ Optical Density (OD) of Negative control].

Statistical analysis

The data collected were analysed using Tukey HSD and correlation, the levels of significance were estimated at $P < 0.05$. The statistical packages used were Excel and Statistical Package

Table 1. Prevalence of urinary schistosomiasis in the study group following urinalysis by microscopy.

Number examined	Number positive (%)	No of male positive (%)	No of female Positive (%)	Intensity (male)	Intensity (female)
173	87 (50.3)	48 (55.2)	39 (44.8)	9.16	11.66

Table 2. Multiple comparisons between different groups of sampled subjects.

(I) ELISA	(J) ELISA	Mean difference (I-J)	Std. error	Sig.	95% Confidence interval	
					Lower bound	Upper bound
Control	Negative	-0.0058	0.01602	0.931	-0.0436	0.0321
	Positive	-0.0363*	0.01510	0.045	-0.0720	-0.0006
Negative	Control	0.0058*	0.01602	0.931	-0.0321	0.0436
	Positive	-0.0305*	0.01201	0.032	-0.0589	-0.0021
Positive	Control	0.0363*	0.01510	0.045	0.0006	0.0720
	Negative	0.0305*	0.01201	0.032	0.0021	0.0589

*The mean difference is significant at the .05 level. Dependent variable: optical density.

for Social Scientists (SPSS) version 11.0 for windows.

RESULTS

The result of the urinalysis by microscopy revealed that 87 (50.3%) were positive for *S. haematobium* of which male subjects had a higher prevalence of 55.2%. (Table 1) The sensitivity and specificity of ELISA in the schistosomiasis positive subjects was 68% and 59.3% respectively. Comparisons between individuals positive and negative for *S. haematobium* revealed a significant difference ($P < 0.05$) between positive subjects and the control subjects, as well as between positive subjects and negative subjects. Conversely, no significant difference ($P > 0.05$) was observed between control subjects and negative subjects (Table 2). There was a significant correlation between IgG titre and age in subjects positive for *S. haematobium*. ($P > 0.05$; $r = 0.5768$) (Figure 1). In *S. haematobium* negative subjects, there was no significant correlation ($r = 0.16$) (Figure 2). In the control subjects there was also no significant correlation ($r = -0.4766$) (Figure 3). However, there was a significant positive correlation between optical density and intensity of infection among the *S. haematobium* positive subjects ($P < 0.05$; $r = 0.612$) (Figure 4).

DISCUSSION

Diagnosis of infection is crucial to disease prevention and

management. Such methods are required not only for people in endemic areas, but increasingly for tourists who may have become infected during visits to such places (Hamilton et al., 1999). Circulating antigen levels (Hassan et al., 1998) and elevations of serum IgE, IgM and IgG (Bout et al., 1980) during schistosomiasis infection have been reported and shown to be good markers in detecting the progress of infection, particularly before and after chemotherapy.

Results from the present study revealed that IgG ELISA had a sensitivity of 68% in schistosomiasis-infected patients. Also our results show that there is a significant difference in the individuals with *S. haematobium* and those without (Table 2), thus showing that this ELISA test actually does differentiate between individual who have been infected and those who have not. There was a significant correlation with age for individuals with schistosome infection in this study (Figure 1), while there was no significant correlation of IgG and age in uninfected individuals and control subjects. This shows that individuals in an endemic area for *S. haematobium* develop antibodies to the parasite over time.

The reason for the 68% sensitivity obtained in this study may be due to the use of the crude egg antigen (Butterworth, 1990) and the polyclonal nature of the antibody. Other workers have reported ELISA specificities of 90.5 and 80% (Salah et al., 2000; Nassr et al., 2002) in *S. haematobium* and *S. mansoni* infection respectively, while we had a specificity of 59.3%. However, results from this study showed that IgG correlated significantly ($P < 0.05$; $r = 0.612$) (Figure 4) to

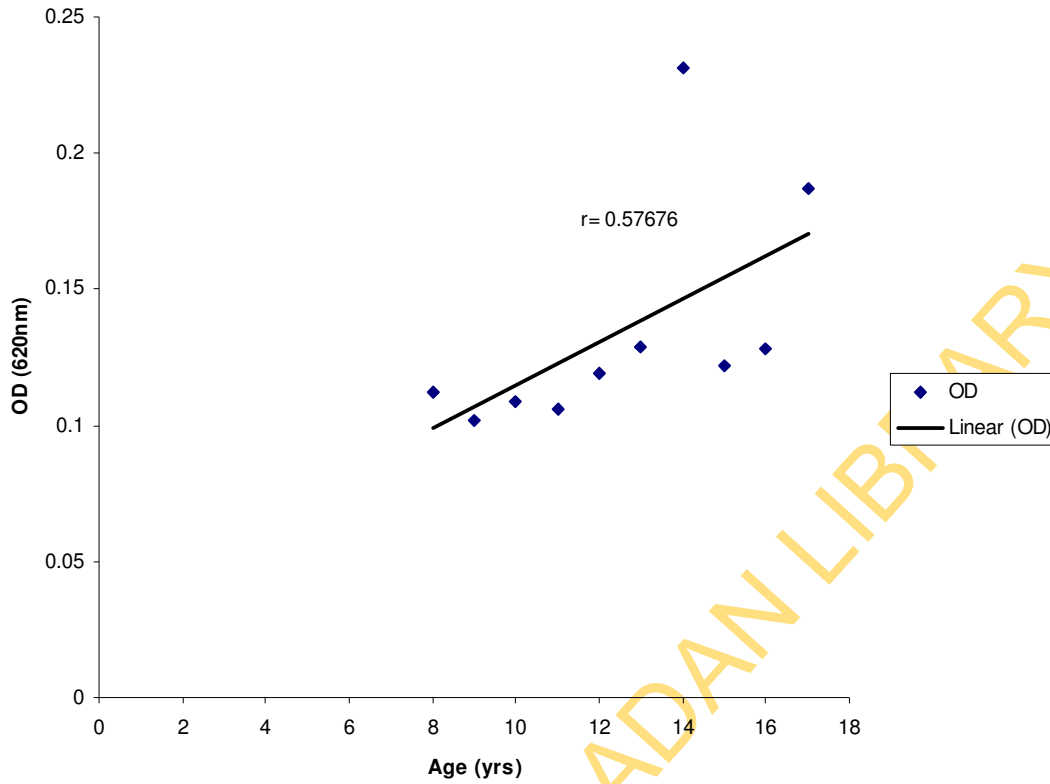


Figure 1. Scatter diagram showing the relationship between age and IgG antibody titre for children infected with *S. haematobium*.

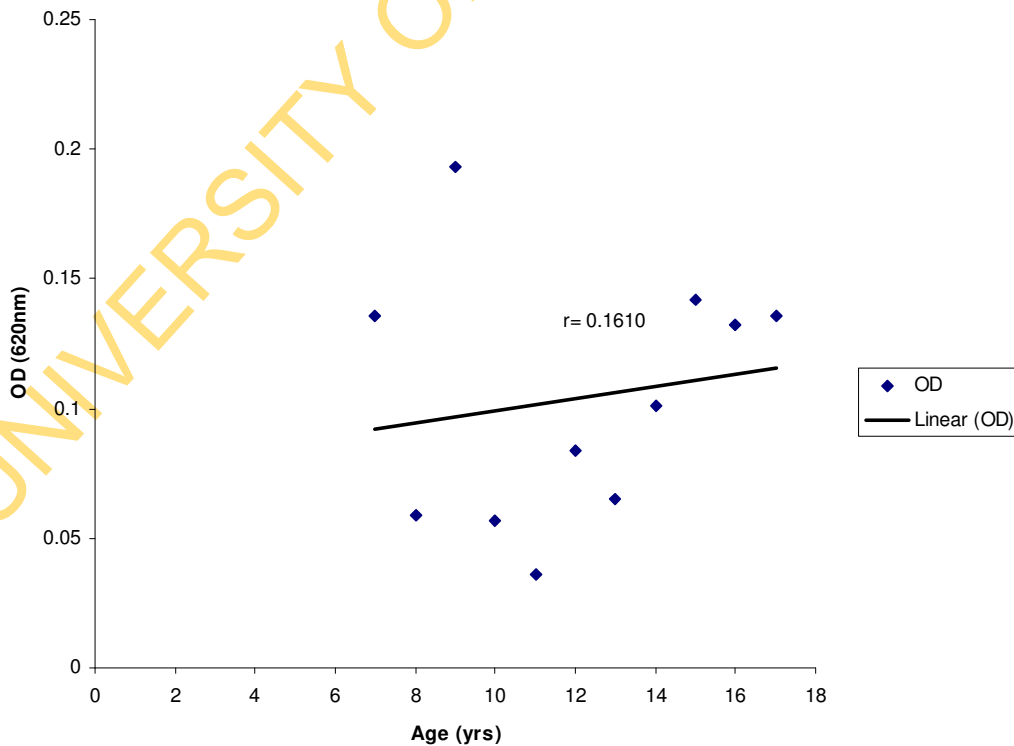


Figure 2. Scatter diagram showing the relationship between age and IgG antibody titre for children uninfected with *S. haematobium*.

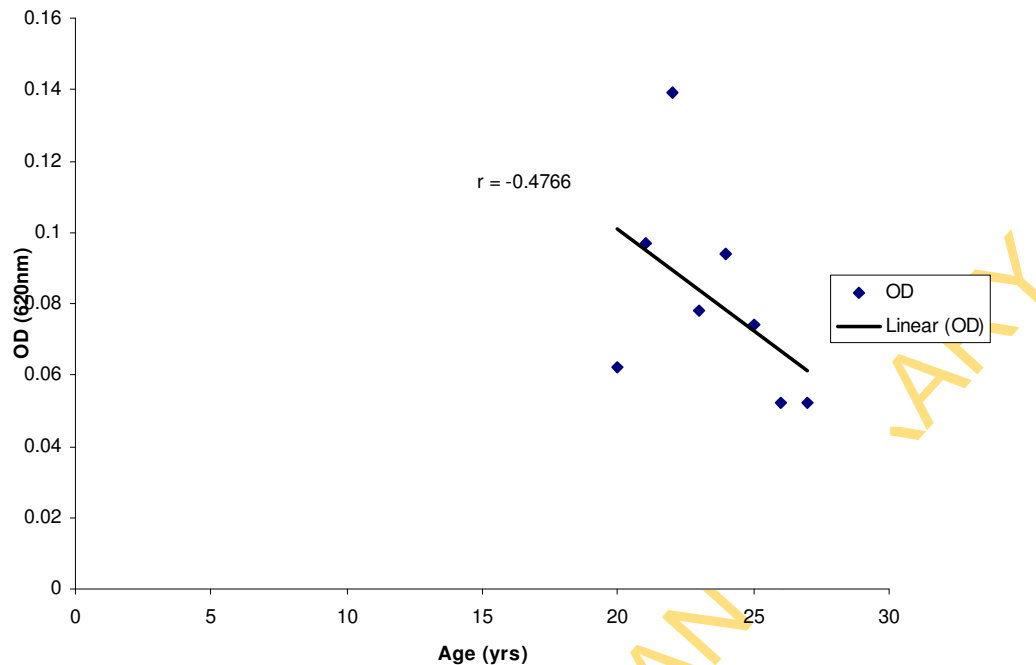


Figure 3. Scatter diagram showing the relationship between age and IgG antibody titre for control subjects.

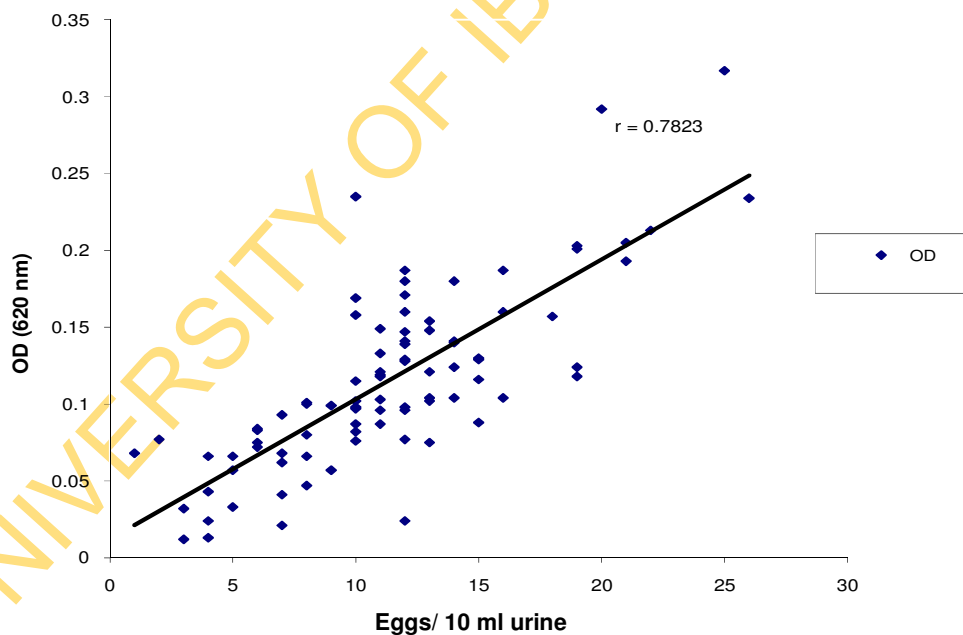


Figure 4. Scatter diagram showing the relationship between intensity of infection and IgG titre in the *S. haematobium* infected subjects.

parasite intensity measured through egg output, with the infected individuals having elevated IgG levels. A previous study had reported that patients with schistosomiasis before treatment had elevated IgG levels (Abou-Basha et al., 2002). Another study had shown

strikingly higher levels of IgG in parasitological positive individuals compared to parasitological negative individuals (Goncalves et al., 2006). Similarly, Stothard et al. (2009) reported sensitivity of 89% and specificity of 70% using SEA-ELISA in detecting urinary schistosomiasis

is in school children. From the foregoing, IgG ELISA has been shown to be an effective immunodiagnostic procedure, particularly in assessing the intensity of infection in the course of the treatment of schistosomiasis.

Results from this study have shown that there was a significant difference, in the IgG of individuals infected with *S. haematobium* compared with uninfected individual and controls. As previously suggested (Hamilton et al., 1999), due to the relative insensitivity of parasitology and antigen detection, antibody detection methods could find increasing use in situations of low infection intensity thus, we intend to improve upon this protocol, and then further compare our results with other test protocols which recorded higher specificity and sensitivity values (Stothard et al., 2009). This is very important as it could then be possible to add IgG ELISA as part of the routine diagnosis of this debilitating disease.

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