

Full Length Research Paper

Assessment of the immunoglobulin status of Nigerians with *Schistosoma haematobium* infection

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The preliminary investigation revealed the prevalence of 138 (46.9%) out of 294 volunteers screened for the ova of *Schistosoma haematobium* in their urine samples. Of these, 84(28.6%) had light infection (50 ova/10 ml urine), while 54(18.4%) had heavy infection (> 50 ova/10 ml urine). This difference was statistically significant at ($\chi^2 = 6.52, p > 0.05$). The mean immunoglobulin status were as follow: IgE (2141.6 ± 143.7 ng/dL), IgG (13.6 ± 3.53 mg/dL), IgA (3.72 ± 0.149 mg/dL), IgM (2.82 ± 0.48 mg/dL) and IgD (0.12 ± 0.04 mg/dL). The relationship between the IgM, IgE and the intensities of infection were positively correlated ($r = 0.27$ and $r = 0.65$, respectively). IgG, IgA and IgD showed negative correlation with the intensities of infection ($r = -0.65, r = -0.39$ and $r = -0.18$, respectively). IgG and IgA can be used as markers of light infection, while IgM and IgE can be used as markers for heavy infection. We deduced that the levels of IgG, IgA and IgM, which were depleted in the infected volunteers, compared to the control subjects, which lacked significant protective effects in these infected volunteers. These low levels of IgA, IgG and IgM and high level of IgE may be involved in maintenance of *S. haematobium* infection in our study area.

Key words: IgA, IgD, IgE, IgG, IgM, *Schistosoma haematobium*, Nigerians.

INTRODUCTION

Schistosoma haematobium, the aetiological agent of urinary schistosomiasis, elicits strong humoral responses in man. For instance, within some endemic areas, infected individuals of all ages have varying levels of circulating antibodies of which the class and subclass composition varies with age and intensity of infection (Jassim et al., 1987; Dunne et al., 1992; Hagan et al., 1991). Also, the data from different geographical settings indicate that the intensities of infection and development of immunological resistance to infections are related in some way to age (Hagan et al., 1991; Butterworth et al., 1985; Butterworth et al., 1991). In Kenya, evidence of the role of IgE among resistant individuals was presented (Dunne et al., 1988). IgA has been implicated in eosinophil-mediated killing of the parasite (Capron et al., 1989).

Human populations living in areas where schistosomiasis is endemic develop antiparasitic antibody isotype responses, which may have distinct roles in immunity to infection and reinfection (Naus et al., 1999). For instance, IgE is associated with resistance to reinfection after treatment, whereas IgM is associated with susceptibility to reinfection after treatment (Dunne et al., 1992). Therefore, insight regarding humoral responses in the pattern of *S. haematobium* infection in any endemic locality is relevant in understanding the pathogenesis of this parasitic infection. In this part of the world, data on urinary schistosomiasis is mainly of an epidemiological nature where factors such as age, exposure and intensity of infection have been advanced to influence the pattern of infection, without considering the immunological implications (Nmorsi et al., 2001; Ukwandu and Nmorsi, 2004; Ekwunife et al., 2004; Nale et al., 2003; Amazigo et al., 1997; Okoli et al., 2006). The existing information is on the cellular immune responses (Nmorsi et al., 2005).

There is a dearth of information on the humoral respon-

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Table 1. Prevalence of *Schistosoma haematobium* infection according to the age groups of the volunteers and the intensities of infection.

Age group in years	Number examined	Light infection (50 eggs/10 ml) no (%)	Heavy infection (>50 eggs/10 ml) no (%)
1-10	24	10 (41.7)	14 (58.3)
11-20	48	16 (33.3)	32 (66.7)
21-30	48	24 (50.0)	8 (16.7)
31-40	56	16 (28.6)	0(0)
41-50	40	8 (20.0)	0(0)
51-60	54	8 (14.8)	0(0)
>60	24	2 (8.3)	0 (0)
	294	84 (28.6)	54 (18.4)
		138 (46.9)	

responses to anti-*S. haematobium* in our locality. To address this problem and add to understanding of the humoral immunological responses to *S. haematobium* infection in our locality, we focused our investigation on the immunoglobulin profile of some volunteers of different age groups in our study area in Nigeria.

MATERIALS AND METHODS

The preliminary investigation took place in Ihieve-Ogben in Owan East local government area of Edo State, Nigeria. It lies at latitude 6°N and longitude 6°E. The characteristics of the study area, the patient population and the community mobilization campaign have been described previously (Nmorsi et al., 2007).

We obtained ethical permission from the state ministry of health, Benin City, Nigeria. All procedures related to this investigation and the purpose of the study was thoroughly explained to the volunteers. Two hundred and ninety four consenting volunteers were recruited for the study. Mid-stream urine samples were collected between 11:00 and 13:00 GMT after slight physical exercises from these volunteers, using a wide-mouthed screw-capped 50 ml container. Their ages were documented individually. These urine samples were transported to our parasitological laboratory for screening for ova of *S. haematobium*. The ova were quantified and classified as light infection (50 ova/10 ml) and as heavy infection (> 50 ova/10 ml), according to WHO standards (WHO, 1983).

The 138 volunteers who had ova of *S. haematobium* in their urine and 60 control subjects that were *S. haematobium*-negative participated in the immunological study. The control participants were volunteers from schistosomiasis non- endemic areas within the same zoogeographical zone. Venous blood was collected individually from both the *S. haematobium*-infected volunteers and the control subjects. The sera were separated by centrifugation using standard procedures. The sera were stored frozen and immediately transported in cold packs to our laboratory for immunoglobulin determination. We used schistosome soluble eggs antigen in our investigation and the levels of schistosome-specific antibodies, namely, IgA, IgD, IgE, IgG and IgM, were determined using the modified radial immunodiffusion assay, as described by Mancini et al. (1965). To rule out intestinal parasites among our studied volunteers, their stool samples were screened using the direct smear and formol-ether concentration techniques. Their peripheral blood were examined using thin blood smears that were stained with Leishman stain to rule out malaria, and HIV infections were excluded from the study group using the Diaspot:Rapid

Diagnostic Test kit (USA), which could affect the immune responses of infected individuals.

The data obtained in this study were subjected to chi-square and Linear Pearson correlation statistical analysis, using the Instat and Microsoft Excel packages.

RESULTS

The prevalence of *S. haematobium* infection according to the different age groups of 294 volunteers and the intensities of infection are presented in table 1. Of the entire group of volunteers screened, 138(46.9%) excreted *S. haematobium* ova in their urine. Heavy infections occurred in 54(18.4%) volunteers, while light infections were reported in 84(28.6%). This difference was statistically significant at ($\chi^2 = 6.52$, $p < 0.05$). The highest intensity of infection was reported among children within the second decade of life. The intensity of infection was light after the third decade of life. Over 75% of the infections occurred amongst volunteers between 1 - 30 years of age.

Table 2 shows the pattern of immunoglobulin status, namely IgA, IgD, IgE, IgG and IgM, among the volunteers who had *Schistosoma haematobium* infection. The mean immunoglobulin profile were as follow: IgG (13.6 ± 3.53 mg/dL), IgA (3.72 ± 0.15 mg/dL), IgD (0.12 ± 0.04 mg/dL), IgE (2141.6 ± 143.7 ng/dL) and IgM (2.82 ± 0.48 mg/dL). IgM and IgE correlated positively with the intensity of infection ($r = 0.27$ and $r = 0.65$, respectively) . The relationship between the intensity of infection and IgG, IgA and IgD were negatively correlated ($r = -0.65$, $r = -0.39$ and $r = -0.18$, respectively). The volunteers within the 11 - 20 age group had the highest IgE (2.380 ± 208.60 ng/dL) and IgM (3.47 ± 0.38 mg/dL) . The highest IgG (17.72 ± 5.01 mg/dL) and IgA (4.00 ± 1.00 mg/dL) were reported in adults of 60 years and above. The mean IgE (2141.6 ± 143.7 ng/dL) and IgD (0.12 ± 0.04 mg/dL) were higher than the control subjects (0 ng/dL and 0.10 ± 0.15 mg/dL, respectively). The control subjects had higher IgG (1255 ± 35.1 mg/dL), IgA (212 ± 11.1 mg/dL) and IgM (110 ± 15.1 mg/dL) than their infected counter

Table 2. The immunoglobulin profile according to the different age groups of the infected and the control volunteers.

Age groups in years	IgE (ng/dL)	IgG (mg/dL)	IgA (mg/dL)	IgM (mg/dL)	IgD (mg/dL)
1-10	2215.2±329.09	7.34±3.59	3.50±0.78	3.27±0.64	0.10±0.06
11-20	2380.00±208.60	11.86±0.92	3.62±0.24	3.47±0.38	0.10±0.07
21-30	2300.01±267.45	10.44±5.36	3.67±0.53	3.20±0.72	0.08±0.07
31-40	2051.01±150.15	15.30±3.51	3.72±0.67	2.60±0.40	0.11±0.08
41-50	2041.00±101.15	15.70±3.01	3.74±0.97	2.60±0.51	0.12±0.04
51-60	2005.02±250.67	16.86±4.96	3.80±0.85	2.58±0.34	0.13±0.01
>60	2000.10±141.05	17.72±5.01	4.00±1.00	2.00±0.44	0.20±0.05
Mean for volunteers	2141.6±143.7	13.6±3.53	3.72±0.149	2.82±0.48	0.12±0.04
Mean for control	0	1255±35.1	212±11.1	110±15.1	0.10±0.15

Table 3. The mean immunoglobulin levels according to light (50 ova/10 ml of urine) and heavy (>50 ova/10 ml of urine) intensities of infection among the infected volunteers.

Immunoglobulins	Light infection	Heavy infection	Mean
IgG (mg/dL)	14.64±3.21	12.56±1.49	13.60
IgA (mg/dL)	3.75±0.39	3.69±0.6	3.72
IgM (mg/dL)	2.27±0.64	3.37±0.71	3.245
IgD (mg/dL)	0.10±0.07	0.15±0.06	0.086
IgE (ng/dL)	2031.0±196.9	2252.2±229	2141.86

counterparts (13.6 ± 3.53 mg/dL, 3.72 ± 0.149 mg/dL and 2.82 ± 0.48 mg/dL, respectively).

Table 3 shows the mean immunoglobulin profile between the light and heavy intensities of infection. The mean IgG (14.64 ± 3.21 mg/dL) and IgA (3.75 ± 0.39 mg/dL) were higher in light infection, and IgE (2252.20 ± 229 ng/dL) and IgM (3.37 ± 0.71 mg/dL) were higher in heavy infection.

DISCUSSION

The data on the preliminary investigation of the prevalence of *S. haematobium*, which indicated 46.9%, can be considered as mesoendemic. Also, the higher prevalence and intensities of infection reported among children within the first and second decades of life reflects the impact of exposure factors on the pattern of *S. haematobium* infection in our study area. For instance, these children have more water contact than adults, as they are involved in both recreational and domestic activities in streams that are their only source of water. This observation is in agreement with the findings of Nmorsi et al. (2007) and Mduluzza et al. (2001). The pattern of infection showed that fewer volunteers had heavy infection. These observations contradict the earlier reports of Nmorsi et al. (2007) in the same zoogeographic zone. Over 75% of the *S. haematobium* infections in our studied locality occurred in volunteers that were 1 - 30 years of age. Similar observations had been documented earlier (Okoli et al., 2006).

We observed a relative higher level of IgE in infected volunteers than in control subjects. Of particular interest is the high IgE elicited by the majority of the volunteers across the different age groups. We deduce that immune mechanisms arising from these antibodies are active at a very young age and these antibodies appear protective, as well as being sustained in older volunteers who are exposed and infected. This deduction agrees with the report of Mduluzza et al. (2001). The high IgE found across all age groups is not surprising considering the role of IgE, which involves fighting parasitic infections. These immunoglobulins can therefore be considered as the major class involved in the humoral immune response to the *S. haematobium* antigens in our locality.

We established a positive correlation between IgM, IgE and the degree of exposure. These antibodies were found to be higher in volunteers within the 1 - 30 year age group than the older adults. Also the data on the intensities of infection and level of immunoglobulins indicated higher IgM and IgE in heavy infection. These classes of immunoglobulin can be used as indicators of heavy and acute *S. haematobium* infection in our locality. Similar reports exist where IgE has been indicated as markers of infection, as it correlates positively with intensity of infection (Hagan et al., 1991; Mduluzza et al., 2001). This information, however, contradicts the data of Naus et al. (1999) whom documented a negative correlation between IgE and the soluble antigen extract of *S. haematobium*. The relatively higher level of IgM found in children compared to adults' deviates from the observa-

vation of Acosta et al. (2004), but agrees with the report of Mduluzza et al. (2001). This is to be expected considering the role they play in blocking other immunoglobulins such as the IgG1 and IgG3, which mediate the killing of schistosomula by human eosinophils *in vitro*. This supports the observation in our present investigation that children and younger adults had more infections than the older adults; a pattern that can be attributed to the level of blocking antibody responses by the IgM, which could prevent the expression of the protective mechanism in these younger age groups (Mduluzza et al., 2001).

Also, we reported higher IgA and IgG in light infection than in heavy infection. Therefore, we can deduce that these classes of immunoglobulins can be used as markers of light infection in our study area.

The depleted mean IgG, IgA and IgM of the volunteers compared with control subjects are of immunological significance. For instance, IgA has been implicated both in eosinophil-mediated killing and anti-fecundity functions in *Schistosoma* infection (Gryzch et al., 1993). We can also deduce that depletion of these immunoglobulins do not make them contribute significantly to protection of the inhabitants to this parasitic infection. The negative correlation between the degree of exposure and antibody levels such as IgG and IgA may further support these immunoglobulins as being important classes involved in the maintenance of chronic *S. haematobium* infection in this locality. These low levels of IgG, IgA and IgM, and high level of IgE are therefore implicated in the susceptibility and maintenance of morbidities of *S. haematobium* in these individuals in our studied locality. Serologically, these levels of immunoglobulins can be used as markers of chronic urinary schistosomiasis in clinical and laboratory practice, especially where clinical presentations of the infection are covert and subclinical.

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