Antibody Response Patterns Against Schistosomahaematobium in some Sudanese Infected Individuals Residents in White Nile State (Sudan)

1 Musa HA, 2 Manahil Nouri, 3* Abdel Bagi AE Fadil (Correspondence Author), 4 Hammad A, 5 Osman MA, 6 Bashir A, 7 Nawal Eltayeb 8 Omer Yasar Hassan, 9 Alfarazdeg A, 10 Mustafa A

1Department of Microbiology National Ribat University, Sudan
2Department of Community Medicine Alneelain University, Sudan
3Department of Immunology, National Ribat University Sudan
4Department of Parasitology, National Ribat University, Sudan
5Department of Microbiology, University of BachtElruda, Sudan
6Department of Microbiology, Khartoum University, Sudan
7Department of Pathology, Khartoum College of Medical Sciences, Sudan
8Sudan Medical Specialization Board, Khartoum, Sudan
9Department of Biochemistry, Khartoum University, Sudan
10 Soba Hospital, Khartoum University, Sudan
elfadiilss@yahoo.com

ABSTRACT

Humans infected with schistosome parasite demonstrate substantial immune responses against both adult worms and eggs. This response can be studied in different age group in both males and females in exposed and infected population. One hundred twenty eight individuals were included in the study. Twenty one subjects who were Schistosomahaematobium negative participated in the study as a control group. Different ELISA techniques were used to detect different anti-Schistosomahaematobium antibodies.

Results: The mean infection intensity was 61.92 eggs /10 ml urine. Peak infections were found among the age group of 3 – 13 year. 53.1% have light infection and 46.9 % have heavy infection. High levels of anti –soluble egg antigen (SEA) IgE was detected in infected individuals in the age range (13 – 23 years) while low levels were observed in individual of >23 year of age. The highest anti – (SEA ) IgA level was detected in old patients. The highest anti-SEA IgM level were found in children aged 3 – 13 year. Females produced high levels of anti-SEA IgE, IgM and IgG, while males produced high levels of IgA.

Conclusion: These results showed high production of IgE which may protect the host until development of other immune responses may also protect the host from reinfection.

Keywords: Immunoglobulin, Schistosomahaematobium.

1. INTRODUCTION

The role of host immunity in the life – cycle, clinical disease and epidemiology of Schistosomiasis has been suggested in several earlier studies. There are at least three separate and distinct aspects of the complex immunologic interaction that takes place between man and this multi cellular helminthes.

The first is immune evasion, which allows developing parasites and adult worms to survive within the human for many years (Maize set al; 1993).The second is the complex immunologic host reaction to the parasite eggs, which is important for egg transport, induces most of the clinical pathology, and is the target of host modulating responses that attempt to destroy trapped ova and yet minimize secondary tissue damage (Donenhoff et al; 1986; Hernandez et al; 1997a). Finally, human chronically infected with schistosome appear to develop partial acquired resistance to new invasions by Schistosomulae (Butterworth, 1998; Capron, 1992). As a result most older humans chronically exposed to schistosomes, appear relatively resistant to new infections (Colley et al; 1986; Butterworth, 1998).

A correlation between the intensity of infection and generation of anti-parasite IgE has been elucidated ( Butterworth et al 1996 ). Th2 cells producing IL – 4 and IL-5 play critical roles in developing high level of anti parasite IgE in Schistosomiasis patients ( Dutra et al; 2002 ). A study in Barzilshowed that adolescents with high resistance to infection by Shistosomamansoni have specific IgE levels that are six to eight fold higher than those with low resistance(Capron et al; 1994 ). Protective immunity against Schistosoma develops gradually with the increase of age ( Hagen et al;1991 ). A comparison of humoralresposes to Schistosomahaematobium in areas with low and high levels of infection in Zimbabwe showed that females produced significantly more anti-SEA IgG, IgG4 and IgM , anti-WWH IgE and IgG1. Individuals from the highly endemic area produce significant more anti-SEA IgE, IgG3 and anti-WWH IgG3 and significantly lower levels of anti-SEA IgA , IgM and anti-WWH IgM, when the effects of infection intensity, sex and age had been allowed for. The age profiles of anti-SEA IgA and anti-WWH IgA and IgM reflect current levels of infection while anti-WWH IgG, IgG2 and anti-WWH IgE rose with age in both areas ( F-Mutapiet al; 1997 ). Another study of age related antibody profiles in Schistosomahaematobium infections in rural community in Zimbabwe showed association between IgE antibody responses and resistance to...
Schistosoma haematobium infection which was indicated by negative correlation between IgE antibody levels and intensity of Schistosoma haematobium infection, and by a positive correlation between IgE responses to SEA and soluble worm antigen (SWA) and age (IP Ndhlouvuet al;1996). The study of total and specific IgE in Schistosomiasis showed high levels of total IgE while the parasite specific IgE was detected in 89.65% of patients. A highly significant correlation was found between total and specific IgE; however, no correlation was found between higher total IgE nor parasite specific IgE or the log and mean of stool egg count (Abdel Azim 1989). Low levels of reinfection ( resistance ) were associated with high levels of specific anti-worm IgE. Specific IgE levels were low in children and reached their highest levels in adults. Statistical analysis revealed that this effect was significant even after allowances were made for age and exposure to infection ( Hagan, et al;1991 ). Studies in immunoglobulin profile in Nigeria, in volunteers infected with Schistosoma haematobium showed that there was positive correlation with the intensities of infection and IgE with concentration of 214.6±143.7 (OPG et al; 2007).

From the clinical point of view the type of parasite - host relationship is most important. It varies from benign commensalism to destructive pathological parasitism. This lead to a wide spectrum of clinical pictures. This may be partly attributed to either different parasite strains or to different host resistance factors or both. It is this last point which triggered this study. Moreover, there is a dearth of information on the Humoral responses to anti-S. Haematobium in our locality. This study was also aimed to assess the immune response (Humoral) to Schistosoma haematobium in people living in a known endemic area in the Blue Nile State.

1.1 Aim of the Study
The study aimed to determine the humor immune responses of Sudanese residents in an endemic area of Schistosoma haematobium.

1.2 Ethical Consideration
Ethical and institutional approval for the study was obtained from the medical research council of the National Ribaat University. Permission for initiation of the study in the area was obtained from the Health Services Director-Elduiem locality. Also a meeting was held with the chief of the targeted village and the objectives and the methods were clearly explained. Written informed consent was obtained from eligible individuals and oral consent was obtained from the participant and parents/guardian before sampling.

2. MATERIALS AND METHODS

2.1 Study Area
The Study was conducted in Hilat Salim village, which is located approximately 15 Km south of Elduiem town in the White Nile state . Water contact sites (White Nile river) are 5 minutes’ walk from all houses. Resident in this area have no access to piped water and depend principally on water from the White Nile and the canal for domestic and agriculture purposes.

The communities of the village are mainly peasant farmers who grow maize, wheat and vegetables. Fishing is carried in the White Nile and temporary pools created by the over flow of the White Nile during the rainy season.

2.2 Collection, Analysis of Urine and Stool
A single terminal urine sample (20-50 ml) was collected in 50 ml container from all individuals participating in the study. The samples were obtained between 10:00am and 14:00 P.m. A few drops of saponin solution were added to samples with visible hematuria to enhance clarity in microscopy (Chebsbroughet al; 1998). The specimens were appropriately labeled with identification numbers and placed in cold box with ice packs. They were processed within 1 - 2 hours of collection in the field. 10 ml was filtered through a 25 mm nucleopore filter (12µm pore size) (Peters et al;1979). The filter was then placed on glass slide and examined microscopically for the presence of Schistosoma haematobium eggs. The intensity was reported as the number of egg / 10 ml urine. The degree of intensity was categorized as light infection (≤ 50 ova / 10 ml of urine ) and heavy infection (> 50 ova /10 ml urine ) according to the World Health Organization standards( WHO 1983 ). To rule out Shistosomansoni eggs and other intestinal helminthes, stool specimens were collected from all individuals who have Schistosoma haematobium eggs in their urine, and processed following the Kato katz procedure (Katz et al;1972). The urine of the participants were aliquoted in cry tubes and stored at -20°C.

2.3 Collection of Blood for Immunological Assay
Five milliliters of venous blood were collected from Schistosoma haematobium infected participants. The sera were separated using centrifugation at 3000rpm for 10 minutes, and then aliquoted in cry tubes and stored frozen at -20°C.

Peripheral blood samples were examined using ICT for malaria with two species device (P.F &P.v) (SD Bio standard Diagnostics PVT-LTD India), to exclude malaria infection. All the participants were offered anti-helminthic treatment with the recommended dose of praziquantel 40mg/Kg body weight after collection of blood sample. Malaria cases were treated according to the treatment regime prescribed by the Ministry of health in Sudan.
Eleven individuals with negative urine samples for Schistosomahaematobium ova and no past history of Schistosoma infection who were neither ill nor under any type of therapy at time of collection were chosen to represent controls from the endemic area. In addition 9 apparently healthy individuals living in a non-endemic area were included in the study as controls from a non-endemic area.

All samples were transported frozen in a cold box to the Parasitology laboratory in the College of Medical laboratory Sciences at the National Riba University and stored at - 80 °C until use.

2.4 Determination of Immunoglobulin E (IgE)

The “DS.EIA.IgE.Total” is a one-step immunoassay, based on the sandwich method. The assay utilizes two high affinity and specificity monoclonal antibodies (enzyme conjugated and immobilized), that can bind to two different epitopes on the intact IgE molecule. The DS.EIA.IgE.Total kit was used for the quantitative determination of total immunoglobulin E concentration in serum of infected individual (128 samples) and controls (12 samples).

2.5 Detection of Immunoglobulin IgA, IgM, and IgG in Sera

Schistosomahaematobium soluble egg antigen (SEA), lot no 281011, freeze dried were obtained through personal communication by purchasing from Schistosoma Biological Supply Centre (SBCS) Theodor Bilharz Institute, Cairo, Egypt. The horse radish peroxides (conjugate) and, substrate (TMB) were supplied from INOVA Diagnostics, Inc San Diego, CA92131. One hundred and twenty seven serum samples from infected individual, in addition to 11 sera from individual in the endemic area and 9 sera from individuals from a non-endemic area were tested for all iso types. The ELISA test based on the original method of Engval and Perlman (1971), was used. Wells of polystyrene micro titer plates (corning costar 9018) were coated with 100 µl of Schistosomahaematobium soluble egg antigen 30 µg/ml in coating buffer (carbonate bicarbonate PH 9.2) per well, then incubated overnight at 4°C. All wells were emptied and washed 5 times with PBS buffer, and blocked by dispensing 300 µl/well of 5% skimmed milk in PBS and then the plate was incubated at room temperature for one hour. After washing three times, 100 µl of serum was dispensed at various dilution 1:100 for IgA and IgG, 1:200 for IgM. The plates were incubated for one hour at room temperature and then washed three time. 100 µl / well conjugate was dispensed and incubated for one hour then washed 5 times. 100 µl of substrate was added in each well. The reaction was allowed to take place in the dark for 20 minutes. The enzyme reaction was stopped by 50 µl of 10 % H2SO4 in each well. The absorbance was measured at 492 nm.

3. RESULTS

The mean and standard deviation of the egg count among infected individual was 61.92 and 57.66 respectively. The prevalence of S. haematobium infection, according to the different age groups of the 128 participant showed that the highest rate fall within the age group 3-13 years (73.43%) followed by the age group 14-23 years (24.61%). The lowest rate was found in those more than 23 year old (1.54%). The gender distribution was 62% males and 38% females. Heavy infection was detected in 68(53.1%) while light infection in 60 (46.8%). The majority of the infected patients were within the age group 3-13 year old. The mean immunoglobulin levels among the infected patients and controls from endemic and non endemic areas were shown on Table 1 and figure 1a, 1b, 1c, 1d. Table (2) shows different immunoglobulin profiles in the three groups (infected, control from endemic and non-endemic area). The level of IgA in the infected and controls from endemic areas showed no significant difference (P = 0.116), while comparison of the level of the infected and controls from a non-endemic area showed highly significant difference (P = 0.000). There was no significant difference in the IgA level between endemic and non-endemic controls (P = 0.092). The level of IgM in the infected and controls from the endemic area showed a significant difference (P = 0.032), and also comparison of the levels of IgM between the controls from the endemic and non-endemic areas was significant (P = 0.053). However the levels from the infected samples in comparison with the non-endemic control showed a highly significant difference (P = 0.000). There was no significant difference in the IgG level between the infected and control groups from the endemic and non-endemic areas (P = 0.746, 0.750 and 0.986 respectively). The highest level of IgE was detected in the age group 13 – 23 (mean value 836.56) (figure 2a). IgA level appear in figure 2b, level was detected highest among the age group > 23 year. The response of IgA appear to increase with age in Schistosoma infected individuals. IgM level are shown in Figure 2c. The level was found to be high within the age group 3 -13 OD (0.23). In case of IgG the highest level was detected among the age group 3 – 13 OD (3.17) (figure 2d). It was found that Females produce high anti-SEA IgE, IgM and low IgA, while males produce high anti – SEA IgA but low levels of other iso types.
Table 1: Anti-schistosoma antibody levels in Sudanese patients infected with *Schistosoma haematobium* and controls

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean &amp; Standarddevation</th>
<th>Concentration</th>
<th>Optical density</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>IgE</td>
<td>IgA</td>
</tr>
<tr>
<td>Infected individuals</td>
<td></td>
<td>Mean 620.4353</td>
<td>.2514</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Std. Deviation 944.4725</td>
<td>.18888</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NO 128</td>
<td>127</td>
</tr>
<tr>
<td>Controls from endemic area</td>
<td></td>
<td>Mean 524.6900</td>
<td>.1621</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Std. Deviation 284.11182</td>
<td>.11084</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NO 12</td>
<td>11</td>
</tr>
<tr>
<td>Controls from non-endemic areas</td>
<td></td>
<td>Mean .0252</td>
<td>.0437</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Std. Deviation .04865</td>
<td>.05505</td>
</tr>
<tr>
<td></td>
<td></td>
<td>N 9</td>
<td>9</td>
</tr>
<tr>
<td>P. value</td>
<td></td>
<td>.728</td>
<td>.001</td>
</tr>
</tbody>
</table>

Figure 1a: Anti-schistosoma IgE levels in Sudanese patients infected with *Schistosoma haematobium* and controls from endemic area

Figure 1b: Anti-schistosoma IgA levels in Sudanese patients infected with *Schistosoma haematobium* and controls from endemic and non endemic area
Figure 1c: Anti-schistosomal IgM levels in Sudanese patients infected with Schistosoma haematobium and controls from endemic area.

Figure 1d: Anti-schistosomal IgG levels in Sudanese patients infected with Schistosoma haematobium and controls from endemic area.
Table 2: Comparison of anti-schistosoma antibody levels in Sudanese patients infected with Schistosoma haematobium and controls.

<table>
<thead>
<tr>
<th>Dependent Variable</th>
<th>(I) Group</th>
<th>(J) Group</th>
<th>P.value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgA OD</td>
<td>Infected</td>
<td>Control from endemic</td>
<td>.116</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Control from non endemic</td>
<td>.000*</td>
</tr>
<tr>
<td></td>
<td>Control from endemic</td>
<td>Infected</td>
<td>.116</td>
</tr>
<tr>
<td></td>
<td>Control from non endemic</td>
<td>Infected</td>
<td>.092</td>
</tr>
<tr>
<td></td>
<td>Control from non endemic</td>
<td>Infected</td>
<td>.000*</td>
</tr>
<tr>
<td>IgM OD</td>
<td>Infected</td>
<td>Control from endemic</td>
<td>.032*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Control from non endemic</td>
<td>.000</td>
</tr>
<tr>
<td></td>
<td>Control from endemic</td>
<td>Infected</td>
<td>.032*</td>
</tr>
<tr>
<td></td>
<td>Control from non endemic</td>
<td>Infected</td>
<td>.053</td>
</tr>
<tr>
<td></td>
<td>Control from non endemic</td>
<td>Infected</td>
<td>.000*</td>
</tr>
<tr>
<td>IgG OD</td>
<td>Infected</td>
<td>Control from endemic</td>
<td>.746</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Control from non endemic</td>
<td>.750</td>
</tr>
<tr>
<td></td>
<td>Control from endemic</td>
<td>Infected</td>
<td>.746</td>
</tr>
<tr>
<td></td>
<td>Control from non endemic</td>
<td>Infected</td>
<td>.986</td>
</tr>
<tr>
<td></td>
<td>Control from non endemic</td>
<td>Infected</td>
<td>.986</td>
</tr>
</tbody>
</table>

* The mean difference is significant at the .05 level

Figure 2a: Comparison of anti-schistosoma IgE antibody levels in Sudanese patients infected with Schistosoma haematobium in relation to the different age groups.
Figure 2b: Comparison of anti-schistosoma IgA antibody levels in Sudanese patients infected with Schistosoma haematobium in relation to the different age groups.

Figure 2c: Comparison of anti-schistosoma IgM antibody levels in Sudanese patients infected with Schistosoma haematobium in relation to the different age groups.
4. DISCUSSION

The relationship between age, intensity of infection and gender has been observed in many epidemiological situations, endemic (Hagan et al. 1998) and epidemic (Stlmaet al. 1993; Van Dam et al. 1996). In the present study, the peak prevalence of infection was among the 3 – 13 year old individuals. This is in accordance with (Onori E et al. 1963; Shimada M et al. 1987). In this study, the rate and intensity of infection were higher in males than in females. Similar observations have been reported from other countries (McCullough et al. 1965; Onori et al. 1963; Chandiwana et al. 1988) with the notable exception of the coastal area of Kenya where adult females were found to be infected more than their male counterparts (Shimada M et al. 1987).

Human infection with schistosoma parasite demonstrate substantial immune responses against both the intravascular adult worms and the schistosoma eggs retained in the tissue. In communities in endemic areas the development of immunity to infection only occurs after many years of exposure. In part this is due to the slow development of antibodies which are protective but also the earlier development of antibody iso types which lack protective capacity and which are capable of interfering with the functioning of protective antibodies (Paul et al. 1992). Protective antibodies appear to be of the IgE class but some IgG subclasses are important. Blocking antibodies were thought to be predominantly IgM and IgG2 but IgG4 also seem to possess blocking activity. The early production of blocking antibodies and late production of protective antibodies may be indicative of cytokine induced immunoglobulin class switching caused by sequential involvement of different lymphocyes. In this study there was high production of all iso types among infected and uninfected residents in the study area compared with the non-endemic residents. This indicates evidence of exposure to the infection. We observed a relative higher level of IgE in infected individual than in control subjects.

Of particular interest is the IgE elicited by the majority of the study population at all ages. This is comparable with that obtained by Abdel-Azim et al. (1989). We deduce that immune mechanisms arising from these antibodies are active at a very young age and these antibodies appear protective. This deduction agrees with the report of Mduluza et al. (2001). These antibodies can therefore be considered as the major class involved in human immune response to the Schistosomahaematobium antigens in our study area.

These antibodies were found to be higher in individual in the age range 3 – 13 years than the older adults. Also the data on the intensities of infection and level of antibodies indicated higher IgM and IgE in heavy infection. These classes of antibodies can be used as markers of heavy and acute infection in the area, and this is similar to the findings of O. P. G et al. (2007). The relatively high levels of IgE in the control group from the endemic area may indicate resistance to infection with Schistosomahaematobium (re-infection). Similar finding were reported by Dunne et al. (1992), when he correlated IgE with resistance to re-infection. The higher level of IgM and IgG found in children compared to adult deviates from the observation of Acosta et al. (2004) but agree with the report of Mduluza et al. (2001). This is to be expected considering the role they play in blocking other
antibodies (IgG1 and IgG3), which mediate killing of Schistosoma by human eosinophils in vitro. This supports the observation in the present investigation that children and younger adults are more prone to infections than the older adults; a pattern that can be attributed to the level of blocking antibody responses by IgM which could prevent the expression of the protective mechanism in these younger age groups. It was also noted that IgA is produced early in the course of infection, therefore suggesting the diagnostic value of these antibody classes. The high levels of IgG in this study was not clear it may be due to blocking antibodies IgG4 or to other subclasses of IgG. So this need further investigation. For all iso types (IgE, IgM and IgG) females produce more antibodies than males except for anti-SEA IgA where males produce more. This is comparable to that reported by Mutupet al, (1997). The differences in iso type level may be due to hormonal differences, although different exposure patterns between the sexes would result in differences in parasite worm burden which may be reflected in antibody levels.

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