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**SCHISTOSOMA MANSONI INFECTION AND THE ASSOCIATED ANTIBODY IMMUNE RESPONSE AMONGST RESIDENTS OF KIGUNGU ENTEBBE, UGANDA**

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**ABSTRACT**

**Background:** There are many foci endemic for *Schistosoma (S.) mansoni* in Uganda. The immune responses to infection with the parasites in these areas have been found to vary with host sex, age and infection intensity.

**Objective:** To determine the profile of antibody isotypes responses against *S. mansoni* crude soluble egg antigens (SEA) and soluble adult worm protein (SWAP) antigens that determine the host resistance or susceptibility to reinfection.

**Design:** Cross Sectional, cohort study.

**Setting:** Kigugu fishing village in Entebbe, Uganda.

**Subjects:** Nine hundred and forty five (945) Kigugu residents reported for pre-treatment screening and enrolment and 626 cohorts report for post-treatment screening and enrolment 18 months later.

**Results:** Pearson's Chi-sq<sup>2</sup> showed that increase in titres of anti (SWAP IgE, SEA IgE, and SEA IgG2) was not significant, but increase in anti SEA IgG3 was significant. Decrease in titres of anti (SWAP IgG1, SEA IgG1, and SEA IgG4) was not significant but decrease of anti (SWAP IgG2, SWAP IgG3 and SWAP IgG4) was significant. Positive correlation existed between age and anti SWAP IgE in before and after treatment sera. On the contrary, age was positively correlated with anti SWAP IgG4 in pre-treatment sera but was negatively correlated with anti SWAP IgG4 in the post-treatment sera. In addition there were positive correlation between higher egg counts and the immunoglobulin levels of anti SWAP IgG4 and anti SEA IgG4 but negative correlations were observed between anti SWAP IgE and anti SEA IgE. Conversely low egg counts were associated with high levels of anti SWAP IgE. Furthermore, IgG1-4, IgE antibody to SEA and SWAP antigens did not differ significantly according to sex.

**Conclusion:** We concluded that praziquantel treatment of *S. mansoni* infected persons alter the immune responses that are influenced by age and intensity. A phenomenon that is useful in the effort to produce vaccine against schistosome.

**INTRODUCTION**

Studying the immune responses of the hosts to *Schistosoma mansoni* infection bridges the gaps in our knowledge about the anti parasite antibody responses to the parasite antigens of people living in the endemic areas. Previous studies from endemic areas in this respect have shown that specific antibody isotype responses against the parasite can promote either

resistance or susceptibility to reinfection (1, 2). High level of IgE antibodies against soluble adult worm protein (SWAP) antigen in the blood is associated with resistance to infection or reinfection while high level of anti SWAP IgG4 is associated with susceptibility to infection or reinfection (2,3). The various antigens in different life stages of *S. mansoni* in the human host elicit multiple antibody responses during the transition period from acute to chronic stages of the

disease (4). In the acute toxæmic phase of *Schistosoma mansoni* infections, levels of specific humoral Ig responses against (SEA) are higher than in (SWAP). Whereas, in chronic schistosomiasis, specific epitope Sm22.6 found in adult worms antigens component is a target for the production of protective IgE. The expression of protective immunity therefore depends on the favourable balance towards IgE production in the chronic infections and IgG 4 produced in acute infection. Beside IgE, mast cells coated with IgG when stimulated with antigens would lead to production of protective antibodies in the entire IgG sub-classes and the release of eosinophils chemotactic factors (4). In endemic areas, periodic treatments with anti-schistosomal drugs have been found to alter immune responses, associated with resistance to reinfection in children above 15 years and adults (2, 5). On the contrary, children between five to ten years continue to be highly susceptible to post treatment reinfections (6). These differences in immune responses in adults and children of endemic areas have been established to be due to high levels of specific IgE in adults that confer resistance to reinfections after treatments (2, 7). Sex has little influence on the production of immunoglobulin but age and intensity of infection have been shown to correlate positively with the immune responses (2). IgE and IgA mediate resistance in adults while IgG2c, IgG4 and IgM are associated with susceptibility especially in young patients (8). IgG4 is known to interfere with IgE combining site on the schistosomulum thus preventing IgE from binding to the high affinity receptor (FcεRI) on eosinophil. In addition, the interference of IgG2c and IgM at the binding sites of IgG1, IgG2a and IgG3 prevent the attachment of these cytophilic immunoglobulins receptor on eosinophils and therefore reduce their defensive role in inducing eosinophil mediating killing of schistosomula (1).

Following the previously mentioned studies, we investigated immune responses of people living in Kigungu fishing village Uganda before and after therapy with praziquantel after 18 months instead of the usual six to one year period used in most of the previous studies (1-3). We still found high level of the antibodies in the patients' sera.

## MATERIALS AND METHODS

**Study area:** This study was conducted in Kigungu fishing village, situated along Lake Victoria in Entebbe peninsula. It is located at latitude 435 to 438 East and 003 to 007 North. It is about 15 kilometres from Entebbe Municipality and about half a kilometre from the Entebbe International airport. This fishing village was selected because it has base line data on prevalence and intensities of *S. mansoni* and other soil-transmitted helminths (9). The population of Kigungu is estimated to be 6,000

people with nearly an equal sex 1:1 ratio. The main economical activity of the inhabitants is fishing with limited subsistence farming mainly for food crops. Their water exposure is high. Hence, the source of infections and reinfections with *S. mansoni*.

**Study population and study design:** An inception cohort of 945 participants of adults and children, residents of Kigungu fishing village were enrolled in this study and evaluated over time for *S. mansoni* infections. Two cross sectional assessments performed within a period of 18 months, one before and other after treatment. The first evaluation included the 945 participants, while only 626 returned for the second evaluation after 18 months. Participants' identifications were based on unique study code numbers, names, sex, age, home locations and the names of head of the families. The immunoglobulins levels in sera taken before and after treatment were correlated with age, egg counts and sex. The age of study patients ranged from 5 to 60 years (mean age was 19 years and median 33.5 years). These were adults who have consented to participate in the study, and children between five and 18 years with parental permission to participate in the study. All registered participants had no anti-schistosomal treatment six months prior this study. We set an outreach clinic, which was used as recruitment centre for the study in Kigungu Primary school. Medical doctors, and nurses, carried out medical examinations and recorded the clinical symptoms, while four experienced Medical Laboratory Technologists carried out laboratory investigations. A social worker undertook recruitments, and explaining the benefit of the study to the participants before and after treatment. The general physical examination included status of the abdominal organs commonly affected by *S. mansoni*. Patients with fever, and body temperature greater than 37.5°C had blood smear test for malaria parasites done. On each working day, approximately thirty patients were enrolled into the study. The daily enrolment was deliberately limited to thirty patients to allow satisfactory processing of the patients' stool samples and treatment of cases the following day. Those patients who were *S. mansoni* egg positive were treated with praziquantel at single oral dose of 40 mg/kg body weight within our study centre.

**Laboratory procedures-** Stool microscopic examination was performed using modified Kato-Katz method as described previously (10, 11) and in following studies. The arithmetic mean of the eggs counted in three slides was recorded as the count in 41.7 milligram of stool. To convert the mean egg counted into egg per gram of faeces a factor of 24 was multiplied by the mean of the eggs counted, {that is, (number of eggs x 1000 mg per g) / (41.7 mg) = 24 x number of eggs/g}.

**Antibody study:** Optimised indirect enzyme-linked immunosorbent assay, (ELISA) system using horseradish peroxidase (HRP) was used to detect and quantify antibody isotypes IgG1, IgG2, IgG3, IgG4 and IgE levels in the sera of 183 participants whose blood samples were taken at the recruitment and 18 months after. Crude extract of soluble adult worm protein antigen (SWAP) and soluble egg antigens (SEA) provided by Centro de Pesquisas Rene Rachou-Fiocruz Laboratory de Immunologia Cellular de Molecular, Av. Augusto de Lima 1715 Barro Preto CEP 30190-002 Belo Horizonte, Minas Gerais, Brazil were used in the study. The surfaces of the wells of microtiter plates (NUNC-IMMUNO™ plate Nalge Nunc International, Denmark), were coated with these antigens diluted in carbonate-bicarbonate buffer, (0.05M, pH 9.6) to constitute 100 ng / 100 µl of antigen per well. The plates were incubated at 4°C overnight to allow the antigen to bind on the surface of wells of the plates (after every subsequent incubation the plates were washed using PBS pH 7.4 containing 0.05% Tween 20 in ELISA washer (812 SW1, SLT No 08139 laboratory instruments Strasbourg-Schiltgheim, France). Later 100 µl of 1% bovine serum albumin (BSA) blocking agent from (Sigma Aldrich Chemie GmbH P.O. 1120 89552 Steinheim Germany 49-7329-979) in buffer (PBS) pH 7.4/ containing 0.05% Tween 20 was added. Plates were incubated at room temperature (RT) for 2.5 hours. Diluted human serum in 100 µl volume (primary/ detecting antibody) was titrated using doubling master titration. Plates were sealed and incubated at 4°C overnight to allow antibody to bind to the antigen in the plates. Anti-human biotinylated mouse monoclonal antibody isotype (secondary antibody) to human sub-classes IgG1, IgG2, IgG3 and IgG4 from Zymed Laboratory, INC.48 Carlton Court So.San Francisco, CA 94080 was diluted at 1:1000 in phosphate buffer saline pH 7.4 containing 0.05% Tween 20. The diluted biotinylated mouse monoclonal anti-human globulins was mixed with Streptavidine conjugated to horseradish peroxidase diluted 1:5000 (ECL Amersham Pharmacia Biotech

AB Inc.800 Centennial Avenue P.O.Box 1327 Piscataway NJ 08855 USA). In addition, 100 µl of the mixture of mouse monoclonal anti-human biotinylated/streptavidine horseradish peroxidase was added to each well in the plate. Plates were incubated at (RT) for two and a half hours and later washed in PBS pH 7.4/ plus 0.05% Tween 20 for five cycles to remove the unbound mouse monoclonal anti-human biotinylated/streptavidine. Chromogen Ortho-phenylene diamine (OPD) (Sigma Chemicals CO. P. O. Box 14508 St.Louis MO 63178 USA 314771-5750), and 30% substrate hydrogen peroxide pre-determined volume according to the total volume require for the test in citric acid buffer pH 5 was added in 100 µl volume to all wells to bind to horseradish peroxidase / streptavidine in the plate. Plates were then incubated at room temperature in a dark cupboard for 30 minutes for colour to develop. After which colour development was stopped using 50 µl of 5M Sulphuric Acid added to each well. The Optical density was measured at absorbance 492 nm in an automated ELISA reader (SLT Sandberg & Schneidewind Stormsweg 5\*22085 Hamburg\* Germany) and the reading printed out using Dot Matrix printer Star Micronics manufacturing UK.Ltd no 423010482251.

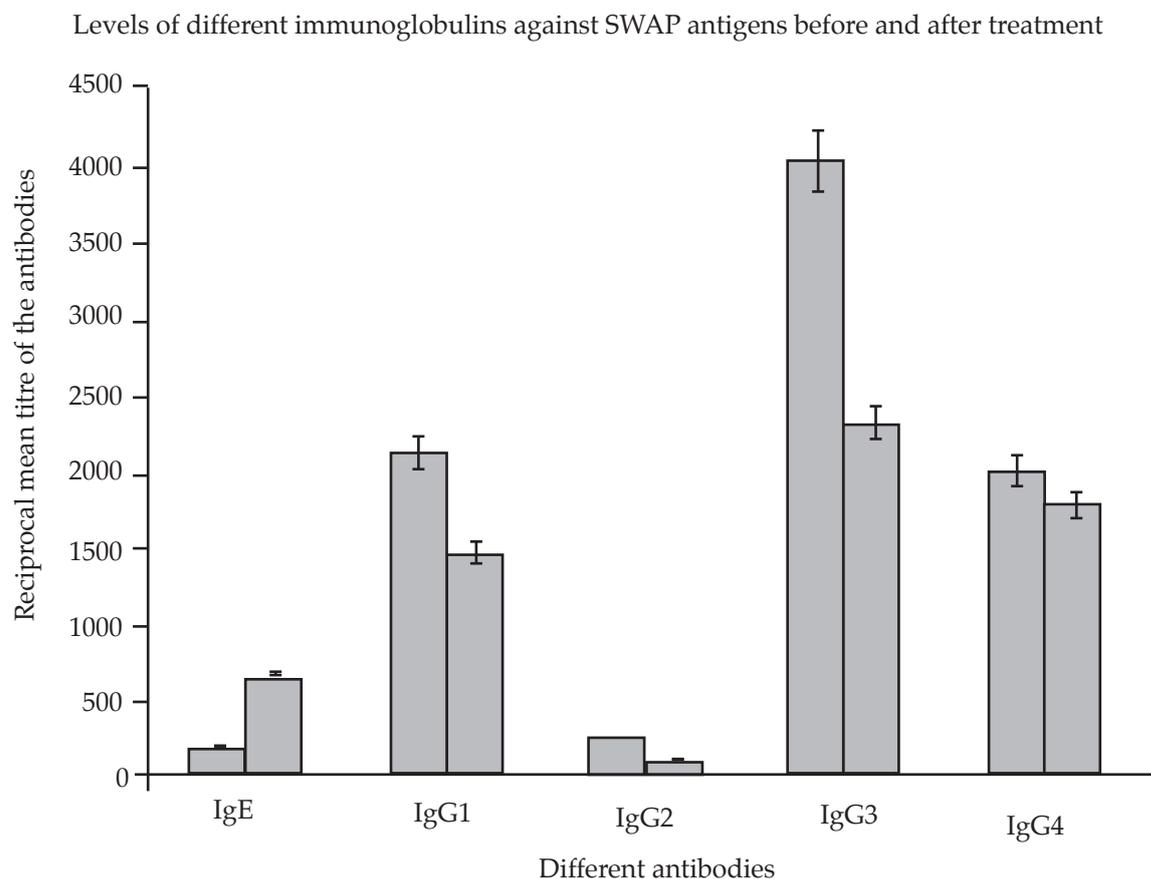
**Statistical Analysis-** There was lack of normality in the frequency distributions of all variables and as well as varying forms of non-linearity in the relationships of specific antibody isotype with age and egg count. Analysis therefore consisted of the following: *Pearson's Chi-sq<sup>2</sup>* was used to test associations between categorical variables; antibody vs. (age, egg count, and sex) data in levels of *S. mansoni* (anti-SWAP or anti-SEA) antibody isotypes in pre and post treatment sera at 95% level of confidence. *Spearman's rank correlation* was utilised to examine quantitative correlations between *S. mansoni* stool egg count, antibody isotypes and age in non-parametric data. All statistical analysis was carried out using SPSSv10, Microsoft Excel, GraphPad software and Stata SE8.

## RESULTS

The reciprocal mean titre of specific antibody isotypes of 183 participants whose blood samples were taken at the recruitment and 18 months after against SWAP antigens are shown in Figure 1.

**Figure 1**

*The mean titre of different antibodies IgE and IgG1-4 against SWAP antigens before and after treatment*



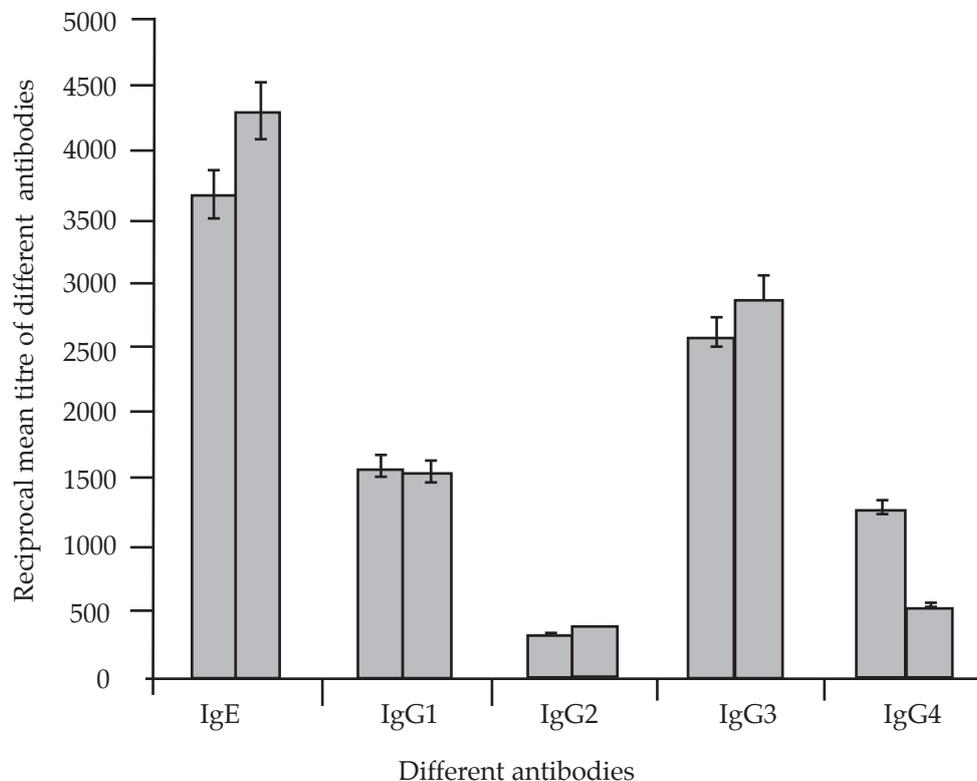
Mean titre of different anti- SWAP among 183 patients before and after treatment. The reciprocal mean titre of anti-SWAP IgE increase from 1: 191 to 1: 660 after treatment was not significant ( $P=0.946$ ). Anti-SWAP IgG1 decreased from 1: 2112 to 1: 1457 after treatment but the decrease was not significant ( $P=0.335$ ). Anti-SWAP IgG2 decreased

significantly ( $P<0.0001$ ) from 1: 261 to 1: 99 after treatment. Anti-SWAP IgG3, also significantly ( $P< 0.044$ ) decreased from 1: 4043 to 1: 2313 after treatment. Anti- SWAP IgG4 significantly decreased ( $P<0.0001$ ) from 1: 2003 to 1: 1778 after treatment. The bars are standard error bars SE at 95% Confident Interval (CI).

**Figure 2**

The reciprocal mean titre of different antibodies IgE and IgG1-4 against SEA antigens before and after treatment

Levels of different antibodies against SEA antigens before and after treatment



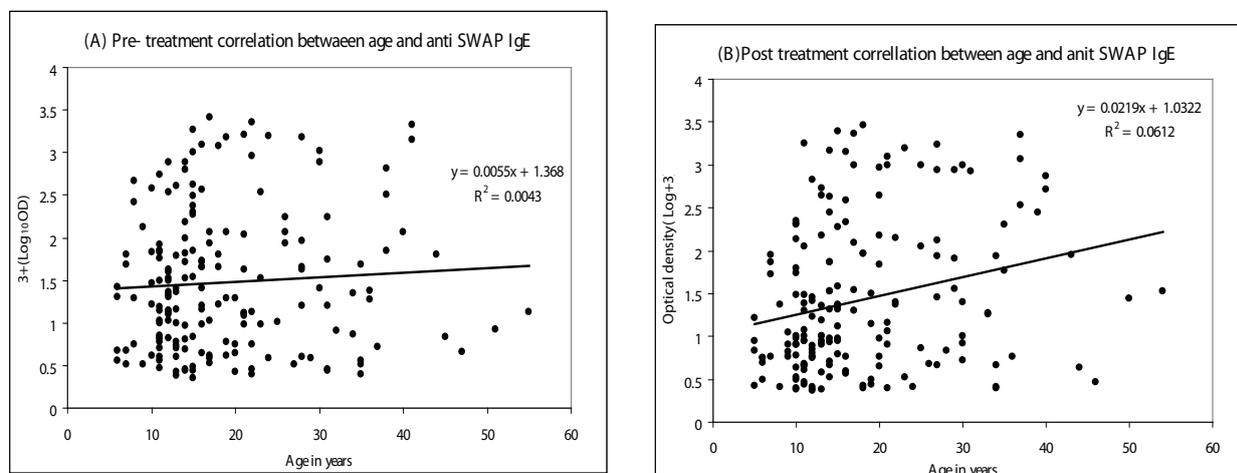
The reciprocal mean titre of specific antibody isotypes of 183 participants whose blood samples were taken at the recruitment and 18 months after against SEA antigens are shown in Figure 2.

Mean titre of different anti-SEA among 183 patients before = and after treatment =. The level of anti SEA IgE remained high after treatment and the titre increased from 1: 3665 to 1:4296 but the increase was not significant ( $P=0.605$ ). Anti-SEA IgG1 decrease

from 1: 1617 to 1:1596 was not significant ( $P=0.116$ ). Anti-SEA IgG2 slightly increased from 1: 330 to 1: 395 but the increase was not significant ( $P=0.199$ ). Anti-SEA IgG3 significantly ( $P < 0.0001$ ) increased from 1: 2621 to 1: 2909 after treatment. Anti-SEA IgG4 also decreased but the decrease was not significant ( $P=0.019$ ) from 1: 1297 to 1: 556 after treatment. The bars are standard error bars SE at 95% Confidence Interval (CI).

**Figures 3**

*The association between anti SWAP IgE and age before and after Praziquantel chemotherapy*

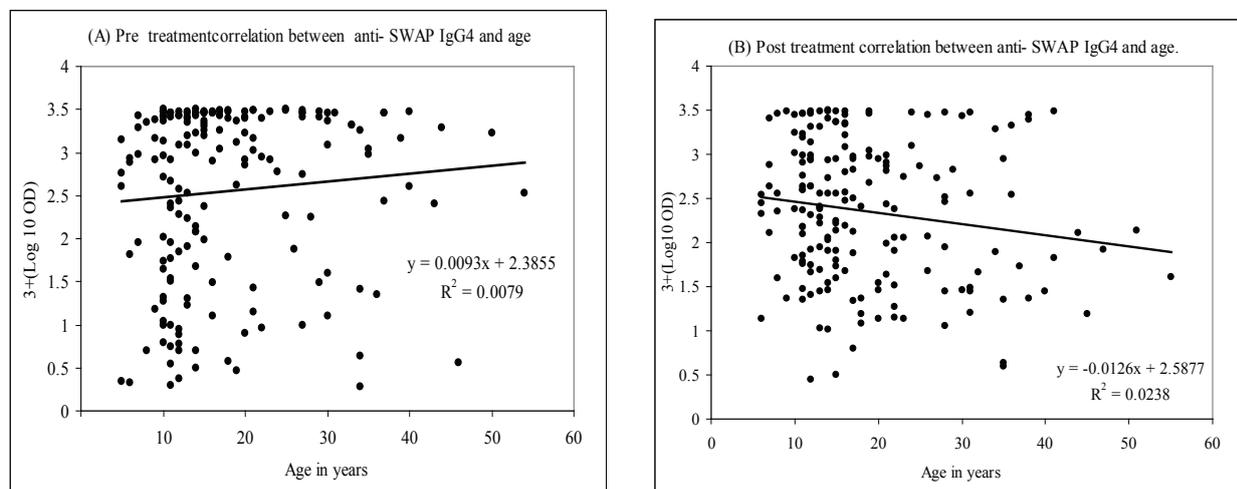


Relationship between anti-SWAP (IgE, IgG4) and age in per and post treatment sera A and B

Key: Sera taken before treatment = A and sera taken after treatment = (B). OD= optical density (3 +Log<sub>10</sub> OD) at 492 nm. y = equation to determine the position of the best line of fit indicated by the black line. R<sup>2</sup> = coefficient correlations measure how strongly the values of x are related to values of y; the levels of anti- SWAP IgE was lower in sera A but was positively correlated to age.

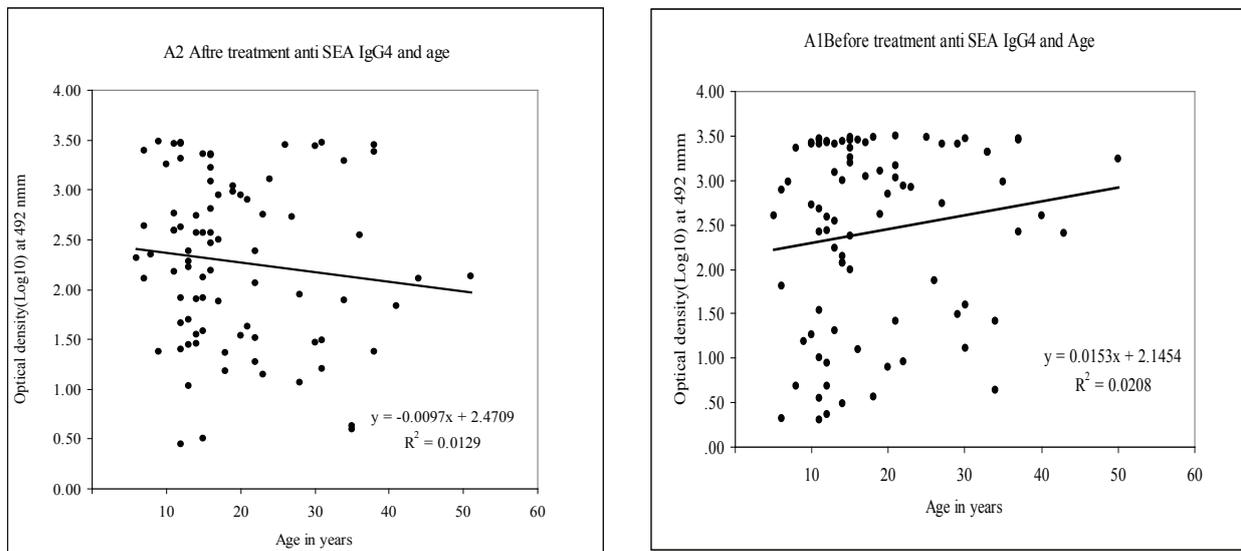
**Figure 4**

*The association between anti SWAP (IgG4) and age before and after Praziquantel chemotherapy*



Key: Sera taken before treatment = A and sera taken after treatment = (B). OD= optical density (3 +Log<sub>10</sub> OD) at 492 nm. y = equation to determine the position of the best line of fit indicated by the black line. R<sup>2</sup> = coefficient correlations measure how strongly the values of x are related to values of y; the level of anti- SWAP IgG4 was negatively correlated to age in sera B.

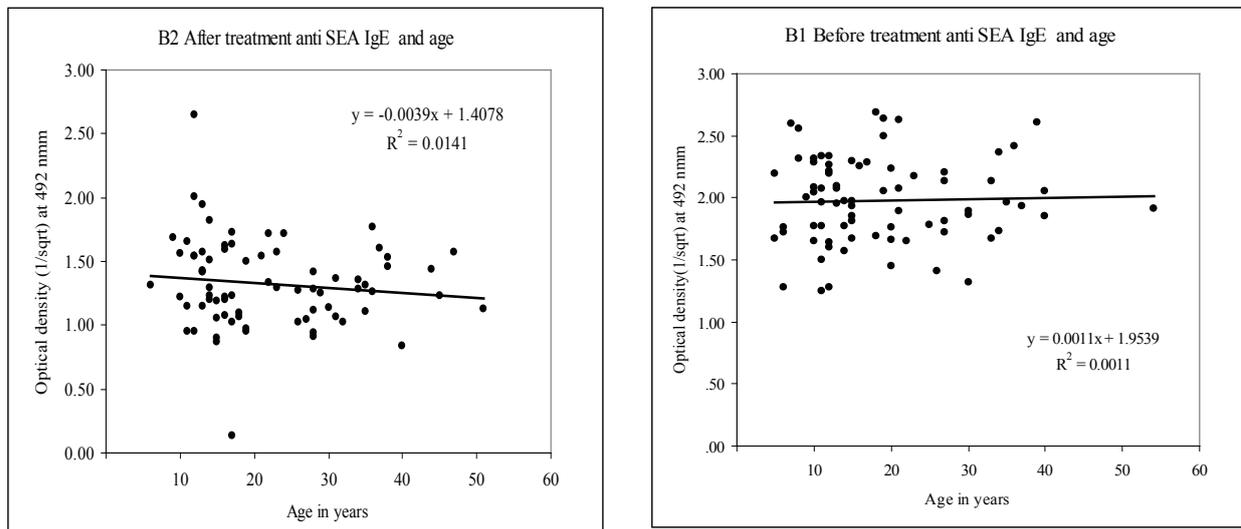
**Figure 5**  
Anti SEA IgG4 and age before and after Praziquantel chemotherapy



Relationship between anti-SEA (IgE, IgG4) and age in per and post treatment sera A and B

Key: A1 and A2=patients with *S.mansoni* before and after treatment; B1=Patient with *S.mansoni* before treatment and B2 are *S.mansoni* negative after treatment. OD= optical density (Log10) at 492 nm; y = equation to determine the position of the best line of fit indicated by the black line; R<sup>2</sup> = coefficient correlations measure how strongly the values of x are related to values of y; · = every dot represents value of absorbance individual serum.

**Figure 6**  
Anti SEA IgE and age before and after Praziquantel chemotherapy



Key: B1 and B2=patients with *S.mansoni* before and after treatment; B1=Patient with *S.mansoni* before treatment and B2 are *S.mansoni* negative after treatment. OD = optical density (Log 1/sqrt) at 492 nm; Y = equation to determine the position of the best line of fit indicated by the black line; R<sup>2</sup> = coefficient correlations measure how strongly the values of x are related to values of y; · = every dot represents value of absorbance individual serum.

## DISCUSSION

Using the optimised indirect (ELISA), Horse radish peroxidase (HRP) system to detect and quantify antibody isotypes in sera of individuals with schistosome, many investigators have demonstrated high level of anti SWAP IgE in the blood of older children  $\geq 15$  years and adults which is believed to confer immunity whereas high level of anti SWAP IgG4 especially in children  $< 15$  is associated with susceptibility to infection or reinfection. These types of immune responses have commonly been observed in *S. mansoni* endemic areas (2-6). Praziquantel treatment of the participants in Kigungu resulted in changes in *S. mansoni* specific antibody responses directed towards soluble adult worm protein antigen (SWAP) and soluble egg antigens (SEA). After treatment, mean titre of serum anti SWAP IgE, increased, while anti SWAP IgG4 decreased. Because of the effector functions of anti IgE and anti IgG4 on host immune responses as demonstrated before in other studies (2, 12), we illustrated association of the same immunoglobulins to age, and intensity.

These changes were largely correlated with age. In Kigungu, children aged between 11-19 years were more infected with *Schistosoma mansoni* than the adults  $\geq 20$  years of age in before and after treatment study. This age group also excreted the higher number of egg than the later in both evaluation studies as have been shown previously in many studies (2, 13). Some of these studies have shown that in *Schistosoma mansoni* endemic areas, there is distinction between age dependent resistance to reinfection after treatment between adults and children (6, 7). In this study it was shown that children in Kigungu in their teens, which peak at 15 years of age, are more intensely infected and reinfected after treatment with the parasite than adults (Figs.3-6). In spite of the fact that water exposures activities to infections in adults in Kigungu are more intense than the children. The current explanation for this phenomenon is that high levels of blocking antibodies like IgM, IgG4 and IgG2c which allow for susceptibility are present in these children's blood (5, 12). Acquired immunity to schistosomiasis *mansoni* in children develops slowly with age owing to decrease in the level of the blocking antibodies and the increase of the amount of antigens in circulations due to frequent exposure to infections (6). As the children accumulate the schistosome antigenic material in their blood over the years, specific antibodies to these antigens also rise in their blood (14).

We observed in the study that anti SWAP IgG4 anti and SWAP IgE was positively correlated with age in pre and post treatment sera respectively

(figs.3,4). In contrast anti SWAP IgG4 and anti SEA IgG4 were negatively correlated to age. Likewise, infection intensity was positively correlated to anti SWAP IgG4 and SEA IgG4. But anti SWAP IgE and anti SEA IgE, was negatively correlated with intensity of infection. Sex was not related to immunoglobulin levels, as was previously reported (2, 15). Resistance to re-infections has been shown to be due to development of high level of anti SWAP IgE. Whereas the levels of anti SWAP IgE and anti SEA IgE increased among the people of Kigungu after treatment the mean titres of anti SWAP IgG4 declined. The decrease of anti SWAP IgG4 and anti SEA IgG4 after therapy was significant. The high titre of most anti SWAP and anti SEA antibodies in sera taken before treatment is due to high amount of worm load and eggs excreted by the female worms in the host initially (2, 15). It has been suggested in other studies that humoral immunity develops slowly against antigens from all life stages of *S. mansoni*. That a 22kDa antigen in *S. mansoni*, which stimulates the production of IgE is localised underneath the tegument of adult schistosome worms and that a good amount of this antigens are released by the dead worms to the immune systems of the hosts after treatment. They further suggested that the increased level of IgG4 antibodies whose production is regulated by the same cytokines, which regulate IgE production, is from the same antigens located underneath the tegument of the adult worms. The activity of the two antibodies is believed to be related, with IgG4 acting as regulator of the allergic anaphylactic responses related to IgE (2, 16). In these studies, the level of anti SWAP IgG4 dropped while anti SWAP IgE increased with age. The phenomenon observed in this study suggests that anti SWAP IgE conferred immunity in older children and adults as was earlier reported (17). However lack of base line data on this type of study in *S. mansoni* endemic areas of Uganda especially this study area to compare with our findings make the interpretation of these findings difficult.

Intensity of infection was independently correlated to different antibody isotypes. Positive correlation between egg counts and immune responses of different antibody isotypes showed that relationship existed between immunoglobulin levels against SWAP, SEA antigens and egg count in before and after treatment. Most of anti SWAP and anti SEA (IgG4) immunoglobulins were positively correlated to egg counts in pre treatment sera except anti SWAP IgE, which was negatively correlated to all egg counts in post treatment sera (2, 13).

In conclusion we conclude that treatment with praziquantel caused changes in the levels of anti

SWAP and anti SEA (IgE and IgG4). Increase levels of anti SWAP, anti SEA IgE have been linked to resistance to reinfections after treatment. Likewise, decrease in levels of anti SWAP, anti SEA IgG4. This study illustrated increased levels of anti SWAP IgE and anti SEA IgE after a single oral dose of praziquantel treatment and decreased in levels of anti SWAP, anti SEA IgG4. It is therefore suggestive that the continuing *S. mansoni* negative observed after a single oral dose of praziquantel treatment in this study, is due to immune responses due to increase IgE and decrease in IgG4. Moreover, we also observed positive correlation between age and anti IgE and negative correlation between age and IgG4 after treatment. In addition, negative correlation between intensity and IgE antibody as opposed to positive correlation between intensity and IgG4 observed in this study further suggest that the resistance or the reinfection observed in this study was influenced by type and amount of antibodies produced by the antigens released from worms and eggs. Sex did not have any influence on the immunoglobulins. The long-term effect of immunological change in Kigungu needs further investigation using specific stage antigens in treatment re-infection study design to establish the nature and development of naturally acquired immunity at short intervals and compare findings with similar studies done elsewhere using stage specific antigens.

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