

## Humoral responses toward cercarial secretions of *Schistosoma Mansoni*: a relationship with age, sex and prevalence of infection

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**Abstract:** Exposure to mercurial secretions induces specific antibody responses, which can be useful to evaluate exposure to *S. mansoni* infection. This paper describes work designed to measure the anti-CTF response (IgG1, IgG4 and IgE) in individuals from a schistosomiasis endemic area of Piida village, Uganda. The predominant anti-CTF antibodies in sera were IgG1 and IgG4. IgG4 specifically recognized antigens at approximately 30 kDa, 46 kDa and 58 kDa molecules. In addition, IgE antibody weakly recognized some molecules of CTF at approximately 22 kDa, 58 kDa and 80 kDa. The effects and the interactions of age, sex, prevalence and intensity of infection on specific antibody levels were also assessed. This study demonstrated that there were different responses in sex dependent age groups. In addition, the anti-CTF IgG1, and IgG4 responses were significantly higher in the age groups of 10-14 and 20-24 years. There was however, no remarkable effect of age on IgE anti-CTF. In addition, there were significant positive correlations between egg-count and the anti-CTF antibody isotypes responses. This study also investigated the relationship between anti-CTF and antibody responses to other *S. mansoni* antigens, including adult (AWA), egg (SEA) and whole cercarial homogenate. Most of these antibodies were strongly correlated with each other. These results suggest that the anti-CTF antibody response appears a reliable indicator of exposure to *S. mansoni* in endemic areas, and might also be exploited for schistosomiasis epidemiological studies.

**Key words:** *Schistosoma Mansoni*, Schistosomiasis, immune responses, ELISA, SDS-PAGE.

### Introduction

Schistosomiasis infection with any of the five species that infect man causes a range of immune-related events at the site of infection in general, and in particular, stimulate antibody production (1). Animal models were often used to study immune responses, but they had many strict limitations. Therefore, recent studies had focused on *in vitro* investigations using sera from people living in endemic areas (2-5). In the endemic areas, the population was exposed to the *Schistosoma* infection from a very early age (6). Researchers pointed out the importance to investigate factors affecting the immunology in these areas such as age, sex

and the intensity of infection (3, 5, 7, 8). There was evidence of earlier changes in the equilibrium of antibodies in more intensely infected populations (9, 10). The protective immunity appeared to increase slowly and the susceptibility to decrease in older children or adults, in spite of evidence that some people were repeatedly infected from a young age (10). It was pointed out that some people were more susceptible to re-infection, while others appeared resistant after treatment for schistosomiasis but the reasons behind these observations were not known (11). The heterogeneous nature of the human exposure to contaminated water was perhaps one

reason; therefore to be able to discriminate between lack of cercarial exposure and acquired resistance would be helpful (5). Several studies reported that the IgG1 and IgG4 were the predominant anti-*S. mansoni* isotypes induced in the sera of infected humans (12, 13). Seroepidemiologic studies in Kenya (14) and in Brazil (15) indicated that the early and high levels of production of IgG4 against adult and egg antigens of *S. mansoni* may block the activity of IgE (16). Accumulating evidence also indicated that the levels of IgE against worm and egg antigen tended to increase with age (3, 5, 17). IgG4 and IgE antibodies had been characterized as markers for developing protection to infection, as well as a risk for immuno-pathology (18). People's contact with water containing cercariae had been extensively studied and had been shown to correlate negatively with age (10, 19).

In *S. mansoni* endemic areas, the intensity of infection peaked between 6-20 years of age and declined rapidly after this age suggesting that the adaptive immune response increased (5, 10). The difference in the intensity of *S. mansoni* infection between the genders from the same community was highlighted, females being with lower intensity of infection than males (3, 20). This was related to different sex behavioural and/or different social culture factors (18, 20, 21). However, a study in mice illustrated that the gender difference might be due to the difference in susceptibility to infection, and due to decreased immunity to infection amongst males (23). Webster *et al.* (7) suggested that the difference in the infection rate between the sexes could be dependent on hormone changes around puberty. The skin penetration process was facilitated by the cercarial transformation fluid secretions (CTF) containing molecules that induce cellular and humoral immune

responses (24, 25). Although, antibody responses had been studied intensively to *S. mansoni* adult and egg antigens (4, 5), schistosomula tegument extract (26) and cercarial homogenate antigens (17), the antibody response to CTF was not investigated in detail. Few studies reported that anti-CTF antibody was considerably more specific than anti-SEA antibody for antibody detection diagnostic test in endemic area (27-29). The main objective of the present study was to measure the antibody response to CTF antigens in humans residing in high endemic area of *S. mansoni* in order to answer the main question: Does anti-CTF antibody responses predict exposure to *S. mansoni* infection?.

## Materials and methods

*Preparation of cercarial transformation fluid (CTF) from S. mansoni:* The CTF that was used for the present experiments was provided by Prof. Mike Doenhoff, University of Nottingham. The material was prepared as follows: *B. glabrata* snails with patent *S. mansoni* infections were placed in distilled water in glass beakers and incubated under a 60 watt tungsten light to induce the snails to shed cercariae into the distilled water. The cercariae were concentrated over a glass fiber filter into a smaller volume of water (approximately 10 ml) and placed in ice to cause sedimentation by gravity. The supernatant was discarded and the cercarial pellet was resuspended in an appropriate volume of PBS, approximately 5 ml PBS per ml of gravity-packed cercariae and the larvae were mechanically stimulated to release the components of their acetabular glands and break off their tails by drawing the suspension through a 20 G needle approximately 15 times. Larval bodies and tails were incubated at 37 °C in a 10 cm diameter plastic Petri dish for 2

hours after which the suspension was centrifuged at 2000 g for 10 min. CTF was collected and stored at - 80 °C.

### ***Human sera and population***

The human sera were kindly donated by Prof Dunne from Cambridge University. Two hundred ninety nine sera, of persons aged 5 to 60 years old and described by Kebatereine and others (30), were kindly provided. The sera were randomly collected from people infected with *S. mansoni* in North-western Uganda. No schistosomiasis treatment had been offered to the population before the sera collection. Parasitological status was assessed by analyzing three stool samples collected on three successive days. For each individual, eggs were counted using the microscope, while personal data was also recorded.

### ***Immunoassay ELISA***

Nine of the 384 well flat-bottom microton 600 high binding plates were coated with CTF diluted in a coating buffer at a final concentration of 5.0 µg/ml. Following the blocking of nonspecific binding sites of the plates with a blocking buffer for 1 hour at room temperature, human infected and uninfected sera were diluted 1: 20 for detecting parasite specific IgE and 1:200 for detecting IgG1 and IgG4 with dilution buffer and incubated overnight at 4°C. The following day, the plates were probed with monoclonal biotinylated mouse anti-human IgG1, mouse anti-human IgG4 and mouse anti-human IgE at 0.5 µg/ml and were diluted with an incubation buffer and incubated for 2 hours at room temperature with gentle shaking. Poly-*HRP* streptavidin complex was added to each plate at a dilution of 1:3000 and incubated for 2 hours at room temperature. After each step described above, the plates were washed with washing buffer 5 times for 15 min. The reaction was then visualized by adding

substrate solution for 10-30 minutes. The reaction was stopped by addition of a stopping buffer; the absorbance was read at a test wavelength 450 nm, and reference wavelength 630 nm. A total of 299 individuals' sera samples were used for measuring antibody responses towards CTF.

### ***Western immunoblotting***

Cercarial transformation fluid at final concentration of 2.0 mg/ml was analyzed by Sodium dodecyl sulphate- polyacrylamide gel electrophoresis (SDS-PAGE), following the method proposed by previously by Laemmli and others. Thus, the CTF molecules of the four sections of the gel were transferred onto a nitrocellulose membrane (NCM). Immunoblotting followed the method developed by Towbin and others. Sera from healthy European volunteers were used as negative controls. Seventeen human sera from the *S. mansoni* endemic area in Uganda were randomly selected for the test.

### ***Statistical analysis***

The statistical analyses were performed using SPSS version 19. The data included sex, age categories and egg-count as independent variables, and immunological parameters (antibody response) as dependent variables. The intensity of infection was analyzed using the Hierarchical Loglinear function. Quantitative analysis of egg-count was done by age and sex based on general linear models (GLIM), and the residuals were indicated if the egg-count was not normally distributed (31-33). The statistical analysis of the effect of sex and age in the prevalence of infection were performed by a full factorial model. The population was divided into 7 age classes (1 = 5-9 years, 2 = 10-14 years, 3 = 15-19 years, 4 = 20-24 years, 5= 25-29 years, 6=30-39 years, and 7= 40-60 years). The intensity of infection

was analyzed in the seven age groups and in two age groups, the age group 20-24 years old and all other age groups as the second age group. A non-parametric model (Kruskal-Wallis test) was used to analyze two or more groups. The Mann-Whitney U test was used to determine variation within two groups. It was further analyzed by categorizing the intensity of infection into 7 groups according to the egg load in the faecal samples. These were: group 1 = 0 egg-count, group 2 = 1-200 egg-count, group 3 = 201-400, group 4 = 401-800, group 5 = 801-1200, group 6 = 1201-2000, and group 7 = 2001-8000.

In order to characterize the relationship between age and IgG1, IgG4 and IgE antibodies specific to CTF from *S. mansoni* infected sera, the individuals were divided into five age groups. The effect of sex, age categories and egg-count on antibody responses were analyzed using GLIM, multivariate and univariate approaches. The distribution normality of 12 immunological

parameters was tested. These parameters were reduced by the Principal Component Analysis into four groups (34). The antibody response to CTF was component 1, response to AWA was component 2 and response to SEA was component 3. The intensity of parasite infections with these principal components was examined for potential effects of age, sex and egg-count, using a non-parametric model (Kruskal-Wallis test). The four principal components were employed as dependent variables and the age (7 levels) and the sex (2 levels) were used as main factors. Pearson's correlation coefficients were used to evaluate the relationships between IgG1, IgG4 and IgE antibody responses to CTF, and between the four antigens of *S. mansoni*. Significance was indicated at the 5% level.

## Results

The study group is described in

of *S. mansoni*. The prevalence of *S. mansoni* infection, in the study population, was very high, 89%.

, including the parasitological characteristics as measured by the antibody responses to CTF

**Table 1:** Description of the study group

Number of Individuals	Age	Sex	Prevalence	Intensity
299 <sup>a</sup>	5/60 <sup>a</sup>	134/165 <sup>a</sup> 47% /55% <sup>b</sup>	266 <sup>a</sup> 89% <sup>b</sup>	0-8226 <sup>a</sup> (1060.24) <sup>c</sup>

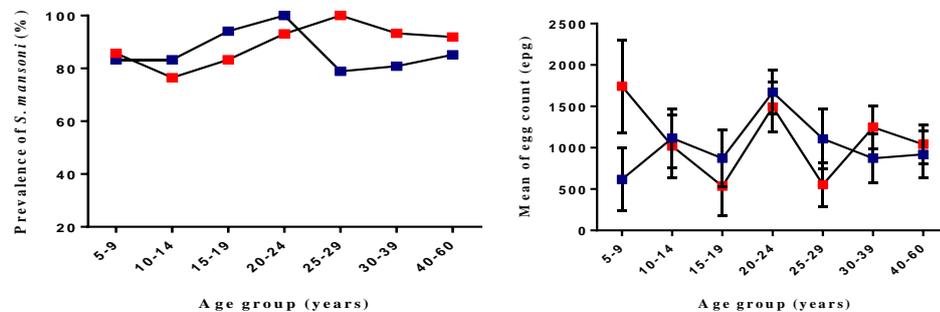
<sup>a</sup>Numbers and ranges of study group, <sup>b</sup>Percentage of infected people (different genders) and prevalence of infections. <sup>c</sup>Mean of egg-count (eggs per gram of faeces) of individuals.

### Age and gender-profiles of intensity and prevalence of infection

Although age was significantly related to the intensity of infection, the parameters of infection of *S. mansoni*: the intensity and the prevalence, did not show any significant differences between the genders ( $P > 0.05$ ). However, females had a slightly higher intensity of infection than males in the (5-9) and (20-24) age groups, and it was higher in the males in the (10-14), (15-19), (20-24) and (25-29) age groups (Figure 1). In both genders, the

intensity of infection was high around the age group of 20-24. The analysis of the intensity of infection in the two age groups (20-24 and all other age groups) confirmed that the parasite egg yield dramatically increased in the 20-24 years old and that during all other age groups the egg-count decreased or stable (Kruskal-Wallis test,  $X^2_1 = 6.194$ ,  $P = 0.013$ ). The prevalence of infection by age and sex (Figure 2) showed a similar trend to that of intensity of infection.

**Figure 1: Intensity of infection by age group and sex**



Red symbols represent the means  $\pm$  SD of females, while the blue symbols represent means  $\pm$  SD of males in each age group (5-9, 10-14, 15-19, 20-24, 25-29, 30-39 and 40-60). The standard error bars show the range of egg-count data, excluding extreme values

**Figure 2: Relationships between age-prevalence of *S. mansoni* and sex in North-western Uganda**





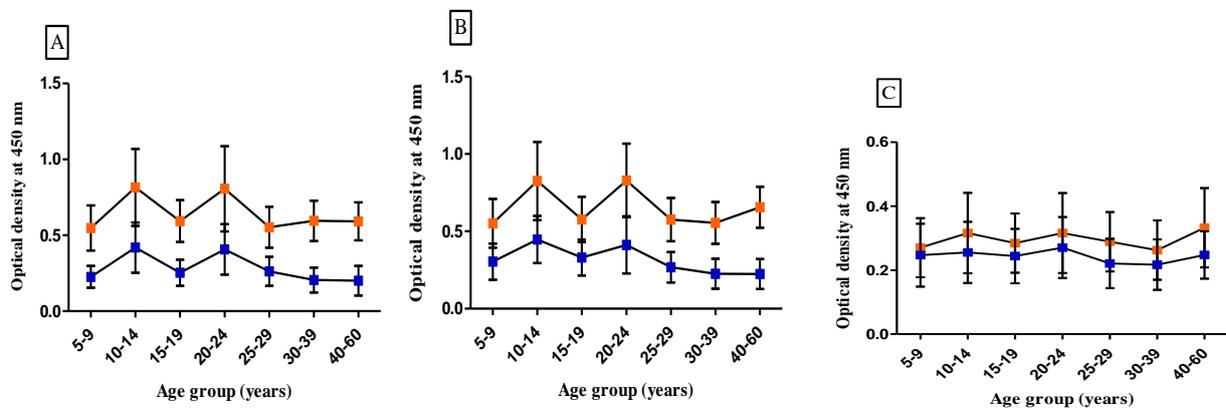
Blue symbols represent males, while the red symbols represent females in 7 age groups (5-9, 10-14, 15-19, 20-24, 25-29, 30-39 and 40-60)

***IgG4 and IgE antibodies specifically recognize antigens of *S. mansoni* CTF by sera of the residents in an endemic area***

Figure 3A characterizes the molecules of CTF recognized specifically by anti-IgG4 and anti-IgE antibodies in the sera from 17

residents infected with *S. mansoni* by the Western blotting analysis. The figure shows that IgG4 antibody specifically recognized antigens with approximately 30 kDa, 46 kDa and 58 kDa molecules. On the other hand, the IgE antibody weakly recognized some molecules of CTF at approximately 22 kDa, 58 kDa and 80 kDa

**Figure 3:** Characterisation of specific antigens of CTF recognised by IgG4 antibody (A) and by IgE antibody (B) from human infected sera



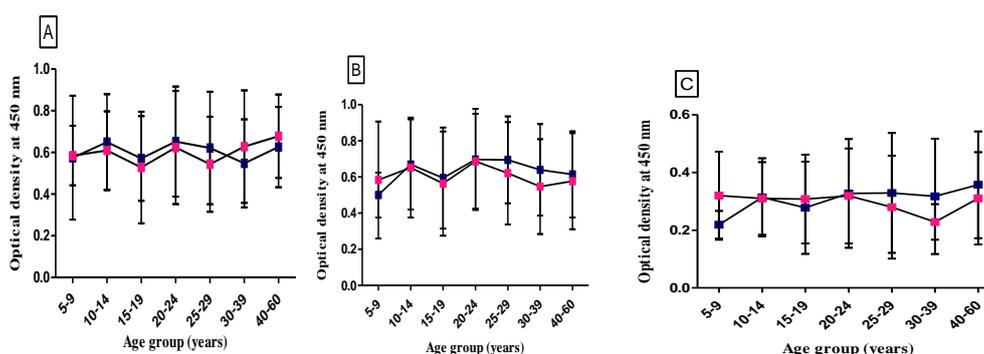
Lanes 1 to 17 represent sera from infected individuals living in a parasite endemic area. Lane 18 represents negative control (healthy European volunteers). Lane MW/kDa presents molecular weights of protein standards in kDa

### ***Specific antibody levels from human infected and uninfected sera in response to CTF***

The mean of different isotypes, anti-CTF IgG1, IgG4 and IgE levels, in the infected and un-infected human sera are shown in Figure 4. The comparison of antibody responses between the age groups demonstrated that for both IgG1 and IgG4

very similar levels were observed in infected and un-infected sera. This corresponded to the prevalence of infection. There was a noticeable increase of antibody responses, particularly in the age groups (10-14) and (20-24), Figure 4. A and B but no such increase was observed with the level of IgE antibody ( Figure 4

**Figure 4:** Distribution of sero-positive of infected and uninfected people sera



Infected sera are represented as orange, squares, and uninfected human sera (Blue, squares) according to antibody levels in each age group (5-9, 10-14, 15-19, 20-24, 25-29, 30-39 and 40-60). The horizontal lines indicate the arithmetic means with SD. A. IgG1, B. IgG4 and C. IgE antibodies response to CTF.

***Relationship of CTF specific IgG1, IgG4 and IgE antibodies with age:*** Comparing the antibody responses between the five age groups, the IgG1 and the IgG4 showed very similar levels (

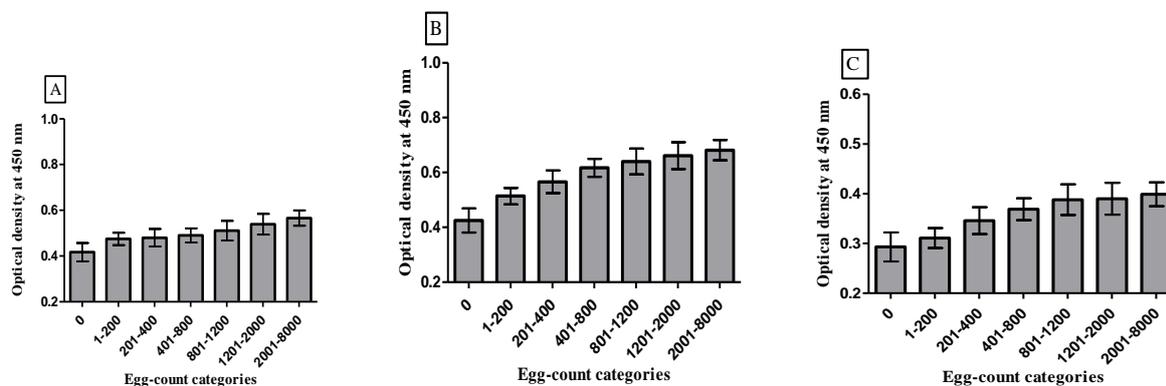
Figure 5 A and B). The specific antibody responses to CTF demonstrated an age related increase over all the age groups with the exception of the 10-14 and 20-24 age groups, which showed remarkable increase in the level of response (

Figure 5A and B). The age profiles of anti-CTF IgG1 and anti-CTF IgG4 reflected the levels of prevalence and intensity of infection. However, anti-CTF IgE responses were low in all the age classes (

Figure 5). The effects of gender on CTF-specific IgG, IgG4 and IgE antibodies were

examined but there was no significant difference in response with sex. Females presented a slightly higher response than males in the (30-39) and (40-60) age groups for IgG1, and in the age group (5-9) IgG4 response was high. In the age groups (5-9) and (15-19) the IgE responses were high. Males were somewhat higher than females in all the other remaining age groups. The IgG4 and IgE responses in males showed increase with age, especially from 20 years old (

Figure 5B and C).

**Figure 5:** Relationship between CTF specific IgG1, IgG4 and IgE antibodies with age and sex

**A.** IgG1, **B.** IgG4 and **C.** IgE antibodies' response to CTF over age profile for endemic *S. mansoni* area; data points are optical density (OD) values at 450 nm. The horizontal lines indicate the arithmetic means with SD. Pink symbols represent females, while the blue symbols represent males.

**Comparison of serum antibody levels towards *S. mansoni* antigens:** Pearson's correlations test indicated a significant positive correlation between the different antibody isotypes (IgG1, IgG4 and IgE) towards CTF (Error! Reference source not found.). Individual responses to CTF, AWA, SEA and cercariae antigens showed positive and highly significant correlations for IgG1, IgG4 and IgE levels ( Table 2: **Characterisation of the relationship between different antibody isotypes to CTF , plus the correlation between levels of IgG1, IgG4 and IgE antibodies to CTF.**

Correlation	Pearson'	P-
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**Table 2:** Characterisation of the relationship between different antibody isotypes to CTF , plus the correlation between levels of IgG1, IgG4 and IgE antibodies to CTF.

Correlation	Pearson's R	P- Value
CTF-IgG1XCTF-IgG4	0.319**	0.000
CTF-IgG1 X CTF-IgE	0.229**	0.000
CTF-IgG4 X CTF-IgE	0.372**	0.000

	s R	Value
CTF-IgG1XCTF-IgG4	0.319**	0.000
CTF-IgG1 X CTF-IgE	0.229**	0.000
CTF-IgG4 X CTF-IgE	0.372**	0.000

Table 3). (r (299) = 0.402, P = 0.000).

**Table 3:** The relationship between different antibody isotypes to some *S. mansoni* antigens

Correlation	Anti-body isotype	Pearson's R	P- Value
CTF X AWA	IgG1	0.441	0.000**
	IgG4	0.239	0.000**
	IgE	0.173	0.003**
CTF X SEA	IgG1	0.542	0.000**
	IgG4	0.114	0.050
	IgE	0.227	0.000**
CTF X Cercariae	IgG1	0.390	0.000**
	IgG4	0.144	0.013

Antibody levels specific for CTF, AWA, SEA and cercariae were measured by ELISA and correlated with one another using Pearson's rank correlation model

### **S. mansoni antigens specific IgG1, IgG4 and IgE antibodies and egg-count**

There were significant positive correlations between the egg-count and the antibody response specific to CTF (Table 4). The increase in the egg-count related to the increase in the antibody response to CTF. The comparison of the antibodies in the 7 egg-count categories and their responses to CTF indicated that all antibody isotypes' responses were higher in individuals with egg-count from 1201-2000 epg (Error! Reference source not found.). The IgG1 levels were significantly higher in the egg-count category 2001-8000 when compared to group 1 (epg = 0) and group 2 (1-200 epg) (Mann-whitney *U*-test,  $z = -2.587$ ,  $P = 0.01$  and  $P = 0.03$  respectively), Error! Reference source not found. A. A significant positive correlation was found between IgG4 antibody responses to CTF and approximately all egg-count categories in individuals with 2001-8000 epg when compared with groups 1 and 2 ( $r(299,$

$0.66) = 0.521$ ,  $P = 0.000$ ), with group 3 ( $P = 0.005$ ) and with group 4 ( $P = 0.015$ ). Group 1 significantly differed with group 4 ( $P = 0.012$ ), with group 5 ( $P = 0.006$ ) and with group 6 ( $P = 0.000$ ), Error! Reference source not found. B. Additionally, anti-CTF IgE response was significantly different between group 1 and the highest egg-count group (Mann-whitney *U*-test,  $z = -2.485$ ,  $P = 0.012$ ), Error! Reference source not found. C). Consequently, principal components (PC1-3) were examined in relation to sex, age groups and egg-count (infected and uninfected). For this, the non-parametric test (Kruskal-Wallis test) was employed; egg-count did indeed significantly change between all the principal components (Table 5). However, in relation to gender, no significant differences were found with all principal components (reflective of antibody responses). Similarly, there was no significant influence in terms of age classes (7 groups and 2 groups) in terms of all principal components.

**Table 4 :** Correlation between egg-count with IgG1, IgG4 and IgE antibody response to CTF

Correlation	Pearson's R	P value
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IgG1	0.216**	0.000
IgG4	0.354**	0.000
IgE	0.159**	0.03

**Table 5:** The effect of sex, age and egg-count in principal components (PC 1-4) of study

#### Eige nanalysis

Compo nent	Initial Eigenvalues			Extraction Sums of Squared Loadings		
	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
1	1.616	53.881	53.881	1.616	53.881	53.881
2	.776	25.867	79.748			
3	.608	20.252	100.000			

Extraction Method: Principal Component

Component Matrix <sup>a</sup>	
	Component
	1
Mean OD CFT IgG1	.679
Mean OD CFT IgG4	.790
Mean OD CFT IgE	.728

Extraction Method: Principal Component Analysis.

Variable	Egg-counta <i>X2, P</i>	Sex <i>X2, P</i>	Age1b <i>X2, P</i>	Age2c <i>X2, P</i>
PC1	10.226, 0.001	0.085, 0.77	7.755, 0.257	0.753, 0.386
PC2	7.689, 0.006	0.159, 0.688	10.163, 0.118	0.933, 0.334
PC3	24.942, 0.000	0.100, 0.752	9.925, 0.135	0.858, 0.354

Principal component 1 is (antibody response to CTF), Principal component 2 is (antibody response to AWA), and Principal component 3 is (antibody response to SEA) a Represents egg-count divided into two groups infected and un-infected individuals. b Age classes characterised into 7 groups, whereas, c represented age into 2 groups. For more information the eigenanalysis and component matrix are presented.

## Discussion

The present study aimed to assess the importance of specific antibody responses to CTF in an *S. mansoni* infected community. In the endemic areas, individuals are frequently exposed to cercariae and their secretions continuously since an early stage in life. This

leads to the production of antibodies against cercarial molecules. It is suggested that IgG4 is a marker to susceptibility to re-infection, whereas, IgE is a marker to resistance to re-infection after treatment (14, 35, 36). It is of interest to determine whether such observations are true for untreated and/or uninfected individual residing in an *S. mansoni* endemic area. In Schistosomiasis

endemic areas, the egg distribution among the infected population is not randomly distributed. However, the intensity of infection is significantly higher in children, with a peak in those between 6-20 years old and then rapidly declining in the adults. The prevalence of *S. mansoni* infection was very high among the population under study. The peak intensity of infection was around the age of 20 years, but there was other obvious peaks followed by a decline in males only. The finding of the current study is consistent with the study carried out in Kenya by Fulford et al. (37). The stated that the peak intensity of *S. mansoni* infection was in the 12-25 years old. Other studies reported that the peak of the intensity of infection, in heavily endemic areas, tends to occur at an earlier age (5-8 years old), and in areas with low level of infection, the peak of intensity of infection tends to occur at an older age (9-20) years old (10, 38, 39). The peak intensity of infection in the females, in this study, was in the age groups (5-9) and (20-24) years old, and the intensity of infection increased significantly from 10 to 30 years as in males. These results were not consistent with the study by Naus and others (3). They reported that males were more heavily infected than females in all age groups. This discrepancy may be due to occupational differences. The mentioned study was performed in an endemic region, where most male individuals were fishermen, who are heavily and repeatedly exposed to infection. The results show that the molecules of CTF induce a number of antibody isotypes in an infected individual's sera, including IgG1, IgG4 and IgE. The percentage of individuals sero-positive within the study group indicates that IgG1 (94%) and IgG4 (83%) are the predominant subclasses in the CTF. These findings are consistent with other studies (3, 13). However, the level of IgE is low (62%). This observation is common in

such communities as a result of diverse susceptibility and resistance to re-infection (14, 15, 40, 41). The comparison of antibody responses between these age groups demonstrated similar IgG1 and IgG4 levels in sera of the infected and the un-infected. These results obviously demonstrate that antibody levels are result and related to the prevalence as well as to the intensity of infection. This study has characterized and identified the molecules of CTF that are recognized by IgG4 and IgE antibodies. The dominant CTF molecule recognized by IgG4 antibody was at 30 kDa. A 30 kDa molecule has also been identified previously as a cercarial elastase (24, 42, 43), and has been suggested as a vaccine candidate (44, 45). Whether the 30kDa antigen recognized here is a elastase is not definite but previous studies did not find any antibody reactivity to any molecule at 30 kDa by Western blot technique (44, 46). However, Pino-Heiss and others (47) reported that a 30 kDa protein reacted with sera, both from infected mice and humans. Further research is needed to investigate and identify the nature of the 30kDa molecule that reacts with human IgG4. Another 22 kDa antigen was also preferentially recognized by IgE. This is most probable to be an allergen-like molecules released from dying *S. mansoni* adult worms and schistosomula (14, 17, 48). The released 22 kDa molecule by the dying schistosomula binds to IgE. The binding results in inflammatory reaction and creates the hostile environment for other invading cercariae (17). An earlier study showed that rat monoclonal IgE strongly reacted with a 22 kDa specific molecule present in schistosomula (49). Future studies are recommended before suggesting that this protein is a promising new biological marker of resistant individuals. The results indicate that the high level of specific IgG1 and IgG4 antibodies responses towards CTF correspond

particularly with the peaks of the prevalence of infection with age groups with the highest percentage of infection (10-14 years and 20-24 years). Previous studies reported that antibody response against AWA and SEA were associated strongly with age and with the intensity of infection (40). This peak of antibodies in these age groups (10-14 and 20-24) is most probably associated with the variation in the individual personal and behavioral period of exposure to infection. The findings suggested a significant positive correlation between egg-count and antibody responses. This supports the reports by previous researchers (3, 7). The analysis by two age groups, the group of 20-24 years old (group 1) and all other age groups (group 2) showed that group 1 is characterized by a significantly higher prevalence than group 2 and that the antibody levels of the individuals of this group are also very high, especially IgG1 and IgG4. There is a strong correlation between IgG4 and the different *S. mansoni* antigens (CTF, AWA, SEA and cercariae homogenate) as well as a positive correlation between IgG4 and IgF responses to CTF. The strong correlation between the IgG4 and the different *S. mansoni* anti-genes is most probably due to that these antigens expressed identical common epitopes, which directly bind to IgG4. Hussain and others (50) suggested that IgE and IgG4 antibodies might bind to the same epitopes. Thus, the effector function of IgE is blocked by IgG4 as they are both directed to the same epitopes (36). However, Li et al. (1999) (51) demonstrated that the two antibodies are independently regulated by different mechanisms. The IgG4 response is significantly different in the different age groups, depending upon the prevalence and the intensity of the infection, whereas no such observation is demonstrated with IgE response to CTF. Also there are no gender differences between IgG1, IgG4 and

IgE responses. However, the levels of IgG4 and IgE increase from 20 years old in the males, while an increase in the levels of IgG1 level is observed from 30 years in females. The IgG4 level is considerably higher in females than males in age 5-9 year old, an observation reported by several workers (3, 7). Two possible explanations were suggested. The first is that males are exposed to cercarial antigens more than females, because of behavioural differences (21). The second is that the difference could be due to different hormonal factors between sexes (7). It will be useful to conduct further studies with a large number of adults over 20 years to assess the cumulative exposure to cercarial secretions, combined with a water contact survey. It is interesting to observe that only egg-count is significantly related to all *S. mansoni* antigen, CTF, AWA, SEA, the principal components. This suggests that the antibody response is a reliable indicator of infection with *S. mansoni* in endemic areas and it might be also exploited for schistosomiasis epidemiological studies. The antibody responses are significantly correlated with the prevalence of infection. The results indicate that IgG4 and IgE responses are associated with sex, age as well as with the prevalence of infection and that anti-IgG4 and anti-IgG1 against CTF increase significantly with the egg abundance. Sera from uninfected individuals, according to negative egg-count results (zero), had seropositive results to CTF antigen with detectable levels of IgG1, IgG4 and IgE. This conclusion is consistent with those of other studies and suggests that such a reaction reflects the limited of microscopic sensitivity of egg combined with a very high sensitivity and specificity of ELISA to detect antibody (52, 53). The sensitivity of antibody detection is a more effective method than parasitology (54).

*In conclusion*, the results suggest that anti-CTF antibody responses predict exposure to S.

*mansoni* but further in depth studies are needed.

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