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Epidemiology of schistosomiasis in Gezira area Central Sudan and analysis of cytokine profiles

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ABSTRACT

Objective: To determine and compare anti-schistosoma IgG, interleukin-10 (IL-10) and interferon- γ (IFN- γ)levels in the serum of patients and endemic controls and to investigate the epidemiological situation of Al-Hebaika village in the northern part of Gezira Agricultural Irrigation Scheme in 2005. **Methods:** During 2005 survey, serum were collected from 118 villagers. Sixty eight were parasitological positive (patients), and 50 were negative (endemic controls). Indirect ELISA was used to measure and compare the levels of immunoglobulin G (IgG) against *Schistsoma mansoni* (*S. mansoni*) soluble worm antigen (SWA) in the patients and endemic control groups from the village and compared with 20 healthy non endemic controls. Sandwich ELISA was also used to measure and compare IL-10 and IFN- γ in the serum of the selected groups. **Results:** The overall prevalence of *S. haematobium* was 20.0% and 0.9% in the first and the second surveys respectively, while the intensity of infection was the same in the two surveys 1.38 [geometric mean egg count (GMFC)]. The overall prevalence of *S. mansoni* infection was 68.5% and 15.4%, while the intensity of infection was 2.75 (GMEC) and 1.70 (GMEC) in the two surveys respectively. IgG reactivity against SWA showed no significant difference between *Schistosoma* positive patients and endemic controls. However, there were high significant differences between each of these two groups and the non endemic control group (P= 0,000). Schistosoma patients and exposed controls had significantly higher IL-10 concentration compared with non endemic controls. While endemic controls showed significantly higher IFN- γ concentration than patients (P = 0.000). Also there was very significant difference between IFN- γ levels of each of patients endemic controls and that of the non endemic controls (P = 0.003). **Conclusions:** The study concluded that IFN- γ has a role in the natural resistant to *schistosoma mansoni* infection. The prevalence and intensity of *S. mansoni* in the Gezira Irrigation Scheme was greatly reduced. *S. haematobium* has disappeared from the area.

1. Introduction

Schistosomiasis is the most devastating parasitic disease of socioeconomic and public health impact in tropical countries. More than 207 million people are infected worldwide in tropical and sub-tropical areas, with an estimated 700 million people at risk in 74 endemic countries. Of the 207 million people with schistosomiasis, 85% live in Africa. The disease affects many people in developing countries, particularly children who are vulnerable to infection[1]. Schistosomiasis is a major health problem in most parts of the Sudan and leads to severe morbidity. The Gezira Agricultural Irrigated Scheme overwhelmed by the high prevalence rates of infection[2]. Human populations living in areas in which schistosomiasis is endemic develop antiparasite antibody isotype responses which may have distinct roles in immunity to infection and reinfection. For example, IgE is associated with resistance to reinfection after treatment and may therefore be involved in protective immunity, whereas IgG4 are associated with susceptibility to reinfection after treatment[3,4]. Cytokines

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also have a major role in the development of pathology and resistance to infection. There are different outcomes that are determined by a balance between different immune responses-modulated by certain cytokines-which are directed both against larval and adult stages of the parasite, as well as parasite eggs trapped in the tissues. interleukin-10 (IL-10) is an important cytokine in regulating the immune response and possibly controlling morbidity in human *Schistosomiasis mansoni (S. mansoni)*, while the production of interferon-γ (IFN-γ) may be associated with resistance to infection[5].

The normal endemic individuals were characterized by their high anti-schistosome antigen response and the lack of infection, determined by the absence of *S. mansoni* eggs in their stool. Furthermore, these individuals also have continuous exposed to the water. Previous studies demonstrate that the NE patients had significantly higher levels of IgE antibodies to tegumental antigen when compared to the treated group resistant to reinfection. Also it was clearly demonstrated that PBMC from NE patients secreted high levels of IFN-γ in response to all antigenic preparations[5]. The NE population constitutes a very important group of individuals who, although living in endemic areas do not get infected. Investigating the immunological factors associated with these individuals is highly relevant for the understanding of immune effector mechanisms involved in the elimination of *S. mansoni* infection and definitely relevant for the development of an effective vaccine. In this study we measure the level of IgG against soluble worm antigen in egg positive patients and egg negative patients which represent as endemic controls and also the concentrations of IL-10 and IFN-γ cytokines in these groups were assessed.

2. Materials and methods

2.1. Study area and population

The Gezira Agricultural Irrigation Scheme (GAIS), in central Sudan, is the largest irrigation scheme in Africa, lies in a triangle of land south of the confluence of the Blue and White Nile Rivers. The total area of the scheme is approximately two million feddans inhabited by 4.5 millions. Villages and agricultural labourers' camps are scattered inside the irrigated area. Al Hebaika village, the study village, is one of these villages which lie in the northern part of the Scheme between two minor canals. The residents of the village are mainly farmers from different Arab tribes.

In 1983, the Tropical Medicine Research Institute, National Centre for Research, carried out a parasitological survey in Al-Hebaika village, in the northern part of GAIS. In March 2005, a census was carried out in the village.

2.2. Study design and data collection

After the objectives of the study were explained to the sheikh of the village and the villagers and their consent to participate in the study was obtained, a cross-sectional immuno-epidemilogical study was done. A census was then carried out in the village in May 2005 to update the 1983 census. Each house was given a household number. All the inhabitants were registered by name, age, sex, occupation, presence or absence of latrines, *etc*. Disposable containers were labeled by specific ID number and distributed to each individual (close relative in case of children) to provide stool and urine samples for schistosomiasis diagnosis.

2.3. Parasitological examination

A parasitological survey was carried out in the village to determine the indices of infection with schistosomiasis. Stool samples were examined by the locally modified Kato method[6]. The results were expressed as egg per gram (epg). Urine samples were examined for the presence or absence of *S. haematobium* eggs by sedimentation technique as described by Braun-Munzinger RA[7]. The results were expressed as eggs per 10 mL. Clinical examination was conducted in the village, by medical doctors, for those who provided stool and urine samples for examination and agreed to participate in the study, as well as those who came complaining from illness but refused to participate in the study. All infected individuals were treated with a single dose of Praziquantel at 40 mg/kg body weight.

2.4. Serum sample preparation

Pre-treatment 5 mL venous blood was collected in plain vacationers from selected groups. The sera were separated by centrifuging at 2 000 rpm for 15 minutes and stored in cryo-tubes at −80 ℃ until use.

2.5. Enzyme linked-immunosorbant assay (ELISA)

Indirect ELISA (Enzyme linked-immunosorbent Assay) technique was used to detect IgG antibody against soluble worm antigen (SWA) in the serum samples of patients and endemic controls as described by Voller *et al*, 1979, with some modifications[8]. The optical density (OD) was measured at 492 nm by ELISA reader laboratory system Multiskan plus (Serial 3140). IFN-γ and IL-10 cytokines were measured in sera of the selected groups by sandwich ELISA using BD OptEIATM ELISA Set B (BD Biosciences, USA, Catalog Number: 550534). All samples and standards with known concentrations were tested in duplicate and the optical density (OD) was read with ELISA reader laboratory system Multiskan plus (Serial 3140) set to 450 nm. A curve was drawn from the standards then from this curve the tests concentrations were calculated in pg/mL.

2.6. Statistical analysis

The data collected during this study was analysed using Statistical Package for Social Science. Where the data were normally distributed or could be normalized, parametric tests were used. Where the data was not normally distributed or could not be normalized non-parametric tests were used. The data of the prevalence and the intensity of *S. mansoni* of the village were analyzed by age groups. *Chi*-square tests were used to detect the significant differences in the prevalence rate of infection between the age groups in the village.

The egg counts were normalized by obtaining the geometric mean egg count (GMEC). One-way analysis of variance was used to determine the significant difference in the intensity of infection in the age groups in the village. One-way ANOVA was used to compare the means of IgG against worm level, IL-10 and IFN- γ concentration.

3. Results

3.1. Population structure

The age structure of the total population in 1983 and 2005 is presented in Table 1. The total population of AL-Hebaika village in 1983 was 1746 and in 2005 was 3187. Although there was a general increase in the percentage in each group, there was a decrease in the younger age groups (5-9 to 25-29 years old).

3.2. Schistosoma haematobium infection

In 1983, the overall prevalence of *S. haematobium* was 20% and decreased to 0.9% in 2005. The intensity of infection was the same in the two surveys (1.38 GMEC).

3.3. S. mansoni infection

There was a highly significant reduction in the prevalence and intensity ($P<0.001$) of *S. mansoni* infection between the two parasitological surveys in 1983 and 2005 (68.5% and 2.75 GMEC compared to 15.4% and 1.70 GMEC, respectively). In 1983, there were no significant differences ($P>0.05$) in either the overall prevalence or the intensity of *S. mansoni* infection between males and females (68.1% and 2.76 GMEC compared to 68.8% and 2.74 GMEC, respectively). However, in 2005, the overall prevalence among males and females showed a highly significant difference ($P<0.001$) (23.6% compared to 8.0%), although the overall intensity of the infection between males and females was not statistically significant ($P > 0.05$), (1.72 GMEC compared to 1.67 GMEC).

There were highly significant differences between the prevalence ($P<0.001$) and the intensity of infection ($P<0.001$) among the different age groups in 1983. Also there were significant differences between the prevalence of infection of the different age groups in 2005 ($P>0.05$), but there were no significant differences between the intensity of infection of the different age groups (Figures 1 a & b).

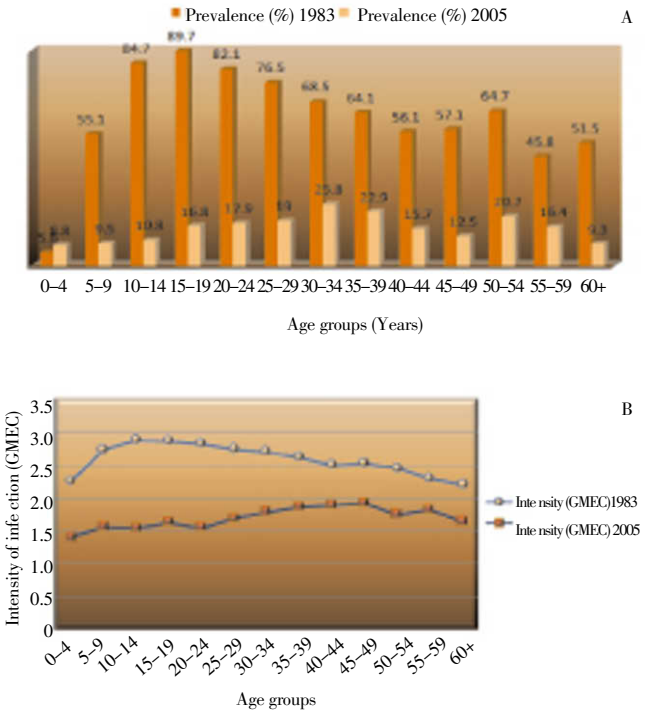

Figure 1. (A) Prevalence and (B) intensity of *S. mansoni* infection by age groups in 1983 and 2005.

Comparing prevalence and intensity of infection between the different age groups in 1983 and 2005, the lowest prevalence was among the age group 0-4 although it was higher in 2005 than in 1983. The highest prevalence in 1983 was in the age groups 10-14 and 15-19 and it was in the age groups 30-34 and 35-39 in 2005. These two age groups were the same groups with the highest prevalence rates in 1983 (Figure 1 A). The highest intensity of infection in 1983 was in the age groups 10-14 and 15-19 while it was in the age groups 40-44 and 45-49 in 2005. The lowest intensities of infection in 1983 were in the older age groups whereas it was in the younger age groups in 2005 (Figure 1 B).

3.4. Parasitological findings

A cohort of 118 individuals was randomly selected to monitor the IgG reactivity against *S. mansoni* worm antigen and the serum Il-10, IFN levels. Sixty eight of the cohort members had detectable schistosoma eggs in their stool during the surveys in 2005 (patients), while 50 individuals living in schistosomiasis endemic area and exposed to the same environment had no detectable schistosoma eggs in their stools in repeated exams. This former group was termed endemic normal and they are considered exposed uninfected

Table 1
Age-gender structure of the population of the village in 1983 and 2005.

Year	1983				2005			
	Males	Females	Total	% age	Males	Females	Total	% age
0-4	50	45	95	5.4	186	169	355	11.1
5-9	134	145	279	16.0	190	169	359	11.3
10-14	163	116	279	16.0	185	187	372	11.7
15-19	136	144	280	16.0	168	165	333	10.4
20-24	91	84	175	10.0	101	113	214	6.7
25-29	76	85	161	9.2	132	141	273	8.7
30-34	47	49	96	5.5	143	136	279	8.8
35-39	40	46	86	4.9	129	134	263	8.3
40-44	37	46	83	4.8	90	118	208	6.7
45-49	14	30	44	2.5	71	78	149	4.7
50-54	33	14	47	2.7	55	67	122	3.8
55-59	20	13	33	1.9	28	45	73	2.3
60+	60	28	88	5.0	90	97	187	5.9
Total	901	845	1 746	100	1 568	1 619	3 187	100

individuals, naturally resistant to schistosomiasis[5].

3.5. IgG against soluble worm antigen

The infected patients and non infected endemic controls had significantly higher IgG reactivity than that found in the non endemic control group ($P = 0.000$). But there was no significant difference in IgG levels between patients and endemic controls (Figure 2).

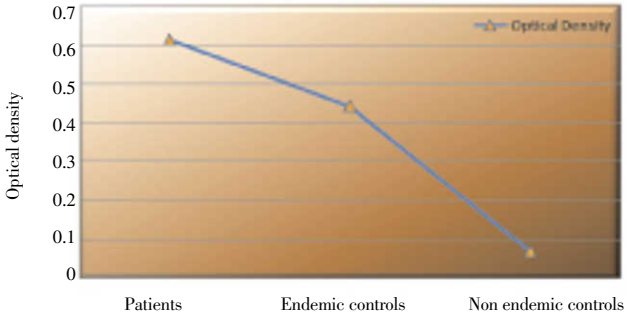

Figure 2. Means of IgG against SWA in patients, normal endemic and non endemic controls.

3.6. IL-10 and IFN- γ concentration

The IL-10 concentration was significantly higher in patients and endemic controls compared with the non endemic control group.

The endemic controls showed significantly higher IFN- γ concentration than active patients ($P = 0.000$). Also there was very significant difference between IFN- γ concentration of each of active patients, endemic controls compared with healthy controls ($P = 0.003$).

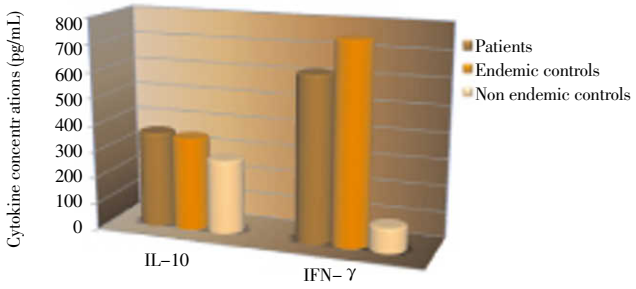

Figure 3. IL-10 and IFN- γ concentrations in patients, endemic and non endemic controls.

4. Discussion

The present study was conducted in Al-Hebaika village in the northern part of GAIS. The village was examined for schistosomiasis infection in 1983. The population of the village was 1 746 (1983 census). 52.2% were males and 47.8% were females and their age ranged between 1-90 years.

The villagers were treated after the parasitological examination in 1983. In addition, the village was offered mass treatment in 1987 as part of the control measures adopted by the Blue Nile Health Project (BNHP) to control schistosomiasis in GAIS. Unfortunately, there was no record about the morbidity of schistosomiasis among the population in the village at that time, or any record about treatment after 1987. The results of 1983 indicated that almost 50% of the population was infected and the egg counts were very high. During the twenty-two years between the first survey in 1983 and the second one in 2005, the population of the village has been doubled. The structure of the population of the village in 2005 showed a general increase in the percentage of each group, but there was a decrease in the younger age groups (5-9 to 25-29 years old). The reason for this decrease is unknown. This observation needs to be confirmed from

other areas in GAIS and the other irrigation schemes before considering it as a phenomenon for investigation.

In spite of this decrease in the younger age groups, the results indicate clearly that the population in the villages of Gezira Scheme is stable. A small proportion, less than 10%, of the population is working outside the country and the natural mortality rate as was expected. The population rarely move to other villages within the Scheme. Such a population would be ideal for study of the development of the disease, the effect of treatment on the endemicity and pathogenesis of schistosomiasis infection and other endemic diseases.

The disappearance of *S. haematobium* from the area is interesting. Although one fifth of the population in 1983 were infected with *S. haematobium*, only 8 individuals were found infected in 2005. However, the epidemiology of urinary schistosomiasis in Gezira Scheme needs more monitoring and evaluation. Nugud reported a similar observation on the disappearance of urinary schistosomiasis from villages in the northern part of the Gezira irrigation scheme[9]. The same observation also was reported from Egypt in the delta of the Nile[10,11].

The reduction of *S. haematobium* among the population of the village could be due to the poor susceptibility of Bulinus snails to the parasite in GAIS after several rounds of mass chemotherapy campaigns or due to an immune mechanism that developed in the human body, or it could be due to a lowered fecundity in old female worms and death of the worms due to repeated treatment, or it could be because the canalization system does not encourage the breeding of Bulinus snails in the canals. However, the end result would be a shift from *S. haematobium* to *S. mansoni* and an endemic *S. mansoni* area.

The significant decrease of both prevalence and intensity of *S. mansoni* infection that was observed in the two parasitological surveys reflects the impact of the control attempts implemented by the national control programs. This improvement may be due to several reasons. Firstly, the residents of Al-Hebaika village were treated several times. In 1983 when the prevalence of infection was 68%, all those who were found infected were treated with a single dose of 40 mg/b.w. of praziquantel. However, in the absence of any control programme the indices of schistosomiasis infection went up to 40% or more. The population in the village was therefore offered mass treatment with a single dose of Praziquantel in 1987, and other supportive control measures were implemented. The control measures adopted were health education, improvement of the sanitary conditions by encouraging the construction of pit latrines and interruption of the transmission by focal molluscicide of the transmission sites. From records, it is clear that treatment of the infected individuals only, in 1983, was not effective unless an integrated programme is also implemented with the treatment. Secondly, the high coverage of the houses with latrine slabs by the BNHP reduced the contamination of the water sources with infected excreta. Thirdly, the presence of adequate safe water supply in the village, which reduced contact with infested water.

In the absence of any control measures adopted in any schistosomiasis endemic area, there are usually no significant differences in the indices of schistosomiasis infection between the sexes. Al-Hebaika village was not an exception, in 1983, the first parasitological survey in the village indicated similar indices of infection in the males and females. This means that both sexes were having the same exposure to cercariae and that all the population in the village was at risk of becoming infected. Twenty two years later, with the advances in the strategies for treatment, the development of effective drugs and the implementation of an integrated control strategy, the differences between the sexes became more apparent in the prevalence but not the intensity of infection. This is mainly due to the activities carried by the males and females in any local community in the GIAS. Although bringing water from the canal is a feminine job, the females do not swim and rarely wash or bath in the canals near the human dwellings; whereas the males usually swim, wash and/or bath in the canals. All the agricultural activities in the fields are mainly carried out by the males. The males' activities require that large skin areas to be exposed to cercariae and thus the difference in the intensity of infection between males and females. It is clear that the type of activity, duration and percentage of the body exposed to cercariae will determine the intensity of infection. The gender difference in intensity of *S. mansoni* infection was reported by several researchers in different countries in the irrigation schemes[12,13].

Generally within any endemic area the prevalence and intensity of *S. mansoni* increase with age to reach a peak in the age groups 10-14 and 15-19 years before they decline in the following age groups (14, 15). The prevalence and the intensity of infection of the population of the village in 1983 showed similar patterns to that found in other endemic areas. The peak of the infection was in the age groups 10-14 and 15-19 years old. However, in 2005 chemotherapy and repeated treatment affect the standard age curves of prevalence and intensity of infection in the village. The highest prevalence was found in the age groups 30-34 and 35-39. These two age groups were the same age groups with the highest prevalence rates in 1983, whereas the highest intensity of infection was in the age groups 40-44 and 45-49. It is worth to mention here that the reproductive age group in GIAS is 30-49 years old. This age group is in a continuous exposure and risk of infection in the absence of the control programmes. Thus, it is reflected in the schistosomiasis indices of infection even among the population treated 22 years before.

The indices of *S. mansoni* infection are affected by

chemotherapy and repeated treatment. The prevalence and intensity of intestinal schistosomiasis in the northern part of GAIS where the integrated strategy of the BNHP was implemented for nearly 10 years is very low in the village under study. However, supportive control measures need to be adopted in the villages to stop the transmission of the disease or reduce it to a minimal level.

Reductions in schistosome infection intensity observed amongst adults in endemic communities are due to acquired immunity to infection. Evidence suggests that this could be linked to changes in the balance of IgE and IgG4 to tegumental antigens[16]. Longitudinal studies of reinfection rates following curative drug treatment have shown that people living in areas where schistosomes are endemic acquire protective immunity after years of exposure to schistosoma infection[17,18]. However, age-related innate resistance mechanisms may also play an important role in the epidemiology of schistosomiasis[17,19].

Children, who get infected, as soon as they can walk, will know before they reach adolescence a long period of susceptibility to multiple re-infections and will represent both privileged targets for the development of the disease and major actors of transmission. It therefore appears that the presence of antibodies response which could lead to the anticipated induction of effector mechanisms reducing the level of re-infection and ideally parasite fecundity would deeply affect the incidence of pathological manifestations as well as the parasite transmission potentialities.

Normal endemic individuals (NE) were described previously by Correa-Oliveira *et al*[5]. These individuals living in endemic areas and have continuous water contact had no *S. mansoni* eggs in their stools and characterized by their high anti-schistosome antigen response. Previous studies demonstrate that the NE individuals had significantly higher levels of IgE antibodies to tegumental antigen when compared to the treated group resistant to re-infection. Also NE individuals had high levels of IFN-γ in response to all antigenic preparations[5]. Post-treatment responses are not similar to those of NE, again suggesting differences in immune protective mechanisms developed naturally vs those acquired after drug treatment.

In this study, the high level of IgG against worm that found in the non infected group indicates clearly that these individuals have an effective immune response. The high IgG against worm level may be due to several reasons. This may be attributed to the possibility that they had an immune response destruct the parasites before their maturation or they had a light infection or they were self-cured, or they had single-sex infection[20]. In fact, they could be actually infected (*ie*. harbour adult worms) but also expressed potent anti- fecundity responses.

The correlation between the outcomes of different clinical forms of this parasitic disease and the establishment of distinct cytokine secretion patterns has not been completely defined. Analysis of cytokines in sera from individuals with different clinical forms, infected or non-infected, is one way to address this issue. Silveira et al. demonstrated that in vitro stimulation with soluble egg antigens of PBMC derived from schistosome egg-negative subjects produced significantly higher levels of the secreted cytokine IFN-γ than the PBMC of individuals whose intensity of infection was determined as more than 100 eggs/g of faeces [21-23]. Similar result was found by de Jesus *et al*[24]. They indicated the role of IFN-γ production in the protective response in human beings by reporting higher levels of this cytokine in partially or completely resistant subjects. The mechanisms involved in parasite destruction in humans are still unclear, as well as the site of parasite killing and other cytokines involved in this process. In mice, IFN-γ-activated macrophages are important in parasite destruction in the lungs[25]. The present study supports the role of IFN-γ production in the protective response in human since significantly higher levels of this cytokine was found in non infected (NE) group when compared with infected one.

IL-10 has clear regulatory roles during *S. mansoni* infection and critically regulates liver pathology[26], and also mediates the control of *S. mansoni* egg-induced inflammation[27]. Although considered a down-regulator of type-2 murine responses, human IL-10 can down-regulate both type 1 and type 2 T-cell responses. In the present study each of the patients group an normal endemic control group have a significant level of IL-10 to regulate their immune responses against the different stages of the parasite.

In conclusion the endemic controls or resistant individuals constitute a very important group who, although living in endemic areas, do not get infected. Analysis of the immune response of these individuals is highly relevant for the understanding of immune effector mechanisms involved in the elimination of *S. mansoni* infection. Studies on this population must continue and must be undoubtedly relevant for the development of an effective vaccine.

The results support the role of IFN-γ in protection against *S. mansoni* infection. The exact role of IFN-γ in protection against *S. mansoni* infection needs more investigations to understand the effector immune mechanisms involved in the elimination of *S. mansoni* infection. The study concluded that the prevalence and intensity of *S. mansoni* in the Gezira Irrigation Scheme was greatly reduced. *S. haematobium* has disappeared from the area. IFN-γ has a role in resistant to *S. mansoni* infection.

Conflict of interest statement

We declare that we have no conflict of interest.

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