PREVALENCE AND IMMUNOLOGICAL ASPECTS OF *SCHISTOSOMA HAEMATOBIUM* AMONG NON-INFECTED FISHERMEN IN WHITE NILE STATE, SUDAN

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Abstract

Bilharziasis is a tropical parasitic disease caused by different species of the genus *Schistosoma*. One species, *S. haematobium,* is widely spread in Sudan. There is a scarcity of information about *S. haematobium* infection among the fishermen in the country. Therefore, this study was designed to determine the prevalence of *S. haematobium* infection among fishermen, and the immune status among non-infected fishermen in two villages, El Hadib and Hawer-Ajowel at the White Nile state in Sudan. A cross-sectional survey was conducted among 119 fishermen in addition to 20 samples from non-endemic area which were included as a control group. Urine samples were examined by sedimentation method and faecal samples were examined by the Kato Katz method and formalin-ether concentration technique. Blood samples were examined for enumeration of IgE antibody and cytokines, interferon Gamma (IFN-γ), Tumor Necrosis Factor (TNF) and Interleukin10 (IL–10) levels using ELISA. The result of the study indicated that the overall prevalence of *S. haematobium* among the fishermen was 35 (29.41 %). Of the 35 positive participants, 23 (65.71 %) were infected for the first time, and 12 (34.29 %) for the second time (re-infection). Twenty-four candidates of the non-infected fishermen were included in the immunological study. The total IgE mean titer was 282.90 ±70.93 iu/ml, IFN-γ mean level was 9.62±4.60 pg/ml. TNF, 114.64±46.63 pg/ml and the IL–10 was 1.51±0.58 pg/ml. High prevalence of *S. haematobium* exists among fishermen and the non-infected fishermen from the endemic area showed high immune response similar to those exposed to the infection.

Keywords: *S. haematobium,* Fisherman, Cytokines, IgE, Immunology, Sudan

Introduction

Schistosomiasis is a disease caused by the parasite of the genus *Schistosoma*. More than 200 million people were infected around the world mostly in Africa (1). Schistosomiasis is one of the three parasitic diseases of public health importance, in term of morbidity and mortality next to malaria (2). The spread of the disease and it endemicity is highly affected by country wide and community water usage, including mechanisms for water conservation, irrigation and generation of hydraulic power (3). *Schistosoma haematobium* and *Schistosoma mansoni* are the major cause of schistosomiasis in Sudan. The disease is endemic in many areas such as Gizera, New Halfa, Rahad, White Nile, and recently in many parts of Khartoum, due to the expansion of water scheme in those areas (4-6).

The disease is usually transmitted among poor communities and many socioeconomic factors affect the distribution of the infection. Among these factors is the frequency of water contact per day (7). Increased water contacts through activities such as bathing, swimming, washing, farming and fishing which lead to increased risk of infection (4, 8). There are many people in the White Nile State who are engaged in fishing. Fishermen are in daily contact with water. They have a greater vulnerability to schistosomiasis and other

parasitic infections and they can also be a source to spread the disease. Although many epidemiological studies of schistosomiasis have been done in different areas in Sudan among farmers, canal cleaner and among school children (4), very few similar studies have been conducted among fishermen who use the water ways. There are many studies that showed the role of immunity in reducing the infection with Schistosoma. The development of antigen–specific immune responses is related to accumulation exposure to parasite antigen and the rate of development of different components determine the outcome of the disease (6). The existing information on cellular responses and humoral responses in Sudan and elsewhere is only on infected individuals, but there is a dearth of information on immune responses in non-infected population in endemic areas (9, 10). The objective of the present study is done to determine the prevalence of schistosomiasis among fishermen in Sudan and the immunological aspect in non-infected fishermen in the study area.

Materials and Methods

Participants and sampling areas

The study was carried out in two villages, El Hadib and Hower-Ajowel in the White Nile state, located at Savanna zone in central Sudan. The study region lies about 321 km South of Khartoum, the capital of Sudan and about 11 km from Rabak; the nearest city. The two villages lie adjacent to the Blue Nile on the east side on the main road to Southern Sudan. The study region experiences a tropical climate with two seasons, the dry season from November to May and the wet season from June to October. The distance from the riverbank to the creek where the nearby village is located is about 3 km. This area is filled with water during rainy season forming an ephemeral lake, which remains for many months.

The population of the area is estimated to be 7,000 according to the report of the last census in 2004. All the participants were male because fishing is only an occupation of men in this region. The men were aged between 14–77 years old (mean 38.5) all having been residents in the area for at least 10 years and doing fishing work for five years at least. They are not included in Sudan government *Schistosoma* control program protocol. The most common problem in this village is lack of basic health and sanitary amenities. The villagers depend on pond water for their domestic use. No human-waste disposal system exists in the villages and the men freely urinate and defecate around the pond (Figure 1).

Figure 1: The villagers depend on pond water for their domestic life where there is no human waste disposal system and they freely urinate and defecate at the pond (left). Fisherman association in El Hadib and Hower-Ajowel village (Right).

The study proposal was approved by the medical ethics committee of The National Ribat University at faculty of postgraduate (Ribat/Eth/PG-01-2016). Information about the study was explained to the participants in the presence of the village chief and also chairman of fishery. To confirm their consent a written or fingerprint document was obtained from participants. All positive cases in the study were treated with a single dose of praziquantel (40 mg/kg body weight). A total of 119 samples were collected from fishermen who were randomly selected and recruited into this study, and 20 samples were collected from participants from Khartoum state (non-endemic area) as control group.

Specimen collection and examination of parasites

Urine and stool specimens were collected from each participant, in two different clean plastic containers which were properly labeled with an identification code number and name. The samples were collected at 12:00 pm to 2:00 pm. Urine samples were examined at the

field on the same day. 10 ml urine sample sediment collected by centrifugation at 3000 rpm and then used to detect *S. haematobium*. The normal saline wet mount of each stool specimens was examined for *S. mansoni* at the field and the remaining stool specimens were preserved in 10 % formalin solution. Then formal ether concentration techniques were employed to process the stool as described by Cheesbrough (11), at the parasitology laboratory, Faculty of Medical Laboratory Science, The National Ribat University. Light microscope was used to detect eggs in urine and fecal samples.

Collection of blood for immunological assays

Twenty-four fishermen negative for *Schistosoma* were selected for testing in the immunological assays and 20 non-endemic controls were included in the assay as healthy controls to be used as reference for cytokines and Immunoglobulin E among fishermen negative for *Schistosoma*. Five milliliters of venous blood were taken from each individual. Sera were separated using a centrifuge at 3000 rpm for 10 minutes. The serum was collected in cryo-tubes and transported in icebox to the research laboratory and stored at -80 ˚C until use.

Detection of total Immunoglobulin E

DS-EIA-IgE total kit (Diagnostic systems Ltd. Russian Federation) was used to detect the IgE in samples from both negative fishermen and non-endemic control following the manufacturer protocol. A Stat Fax–2600 Microplate washer (Awareness Technologies; United State) was used to wash the microtiter plate and the microplate reader Stat Fax 4200 was used to read the test plate.

Cytokines detection

Assays for cytokines were performed by ELISA using commercial cytokine-specific monoclonal antibody pairs according to manufacturers' protocol. The cytokines assayed from the frozen serum were Interleukin 10 (IL-10), Tumor necrosis factor (TNF), and Interferon-gamma (IFN-γ). Ninety-six well ELISA plates (Corning Costar 9018) were coated with 100 µl of monoclonal antibody diluted in phosphate buffered saline (PBS) and incubated overnight at room temperature. They were then washed three time (ELx405 Microplate washer, Biotek) with 0.05 Tween 20 (v/v) and blocked with PBS plus 10% bovine serum albumin (BSA) at 300 µl per well for 1 hour with shaking. The plates were then washed 4 times. The serially diluted cytokine standards, 100 µl were added into respective wells. Undiluted serum samples, 100 µl were added per well followed by 2 hours incubation at room temperature with shaking. The plates were then washed 4 times with wash buffer and the appropriate monoclonal antibody (detection antibody) was added at 100 µl per well for another one-hour incubation at room temperature. This was followed by another 4 times wash before addition of 100 µl diluted streptavidin-horseradish peroxides conjugate. After 30 minutes incubation at room temperature, 5 washes were performed. Tetramethylbenzidine peroxides substrate (TMB) was added at 100 µl per well and colour development at room temperature was allowed for 20 minutes. Stop solution (1 N sulphuric acid) was then added at 100 µl per well. The optical density of each well correlated to cytokine quantity was determined immediately using an automated microplate reader (Stat Fax 4200) at 450 nm.

Statistical analysis

The data were analyzed using SPSS version 16, USA. Statistical significance was considered at $P<0.05$.

Results

The total number of the study population was 119 individuals. The age ranges from 14 to 77 years old (mean 38.5). The majority of the participants were 30–50 years of age and most of them were illiterate. All the participants provided urine and stool samples. No case of *S. mansoni* was detected among the study population. Thirty-five (29.41%) were positive for *S. haematobium.* Of the 35 positive participants, A total of 23 (65.71%) were infected for the first time based on participant history data on questionnaires, whereas 12 (34.29%) were infected for the second time (re-infection). Based on participants data collected in questionnaire; a total of 48 (40.34%) participants had a past history of *S. haematobium*. Hence the number of the non-infected fishermen was 36 (30.25%). The negative group did not receive any anti-*Schistosoma* treatment.

The pattern of IgE level among the non-infected fishermen from endemic areas of *S. haematobium* and control from non-endemic is shown in Table 1. The mean and standard deviation of IgE levels was 282.90 ± 70.93 iu/ml among non-infected fishermen from endemic areas and 72.10 ± 50.63 pg/ml in control group from non-endemic area. The range of the level of IgE in those non-infected fishermen and control from non-endemic area were 244.57 and 199.26 iu/ml respectively. Table 1 also shows the cytokine profile in serum of non-infected fishermen and control group of non-endemic area. There were high levels of TNF and IFN-γ detected in fishermen than in the control group, with high significant difference respectively. IL–10 level was slightly higher in the fishermen than the control group. The difference was also significant ($p=0.01428$). Table 2 shows the correlation between the IgE and different cytokines in the non-infected fishermen from endemic areas. They were all negatively correlated.

Table 1: Cytokine & Immunoglobulin E profile in the sera of Sudanese fishermen from endemic area of *Schistosoma haematobium* and control from non-endemic area

Cytokine/ Ig	Groups	Range	Mean±std	P value
IFN-γ (pg/ml)	non-infected fishermen	17.00	9.62±4.60	0.0114*
	non-endemic control	8.92	6.55±0.25	
TNF (pg/ml)	non-infected fishermen	227.36	114.64±63.00	0.00001*
	non-endemic control	93.41	70.19±21.39	
IL-10 (pg/ml)	non-infected fishermen	2.08	1.51±0.58	0.01428*
	non-endemic control	1.36	1.16±0.36	
IgE (iu/ml)	non-infected fishermen	244.57	282.90±70.93	0.00001*
	non-endemic control	199.26	72.10±50.63	

* Statistically significant

Table 2: Correlation between IgE and different cytokines in non-infected fishermen from endemic areas

	Cytokine	P value	R value
IgE	IFN- γ	< 0.00001*	-0.2644
	TNF	< 0.00001*	-0.0142
	IL-10	< 0.00001*	-0.3921

* Statistically significant

Discussion

Fishermen community is the most common groups who are frequently in contact with water and commonly exposed to water borne diseases, especially schistosomiasis. The fishermen potentially represent a primary source of *Schistosoma* infection. The present study provides baseline information about the immune status of the fishermen free of schistosomiasis. It was reported in previous study that the prevalence of *S. haematobium* in White Nile was higher than the recorded prevalence among community members other than fishermen in the same area 21.4% (12).

Different prevalence was recorded by many researchers in different countries among the fishermen community, including 16.35% In Burkina Faso (13), 13.9 % in Alagoas (14), 29.21% in Ethiopia (15), 47.4% in Uganda (16) and 47.85% in Tanzania (17). The host immune responses, humoral and cellular responses are activated following the exposure to the parasitic antigens (6). The specificity and level of immunoglobulin in schistosomiasis are affected by geographical origin of the host, duration, intensity of the infection and age of the individuals.

IgE plays an important role against infection with the parasite. In this study the total IgE was found to be very high and these observations are in an agreement with a previous study conducted by Capron and Capron (8) on schistosomiasis among non-infected individual. In post treatment, high levels IgE were reported in few studies (18-22) among participants exposed to repeated *Schistosoma* versus first time susceptible individuals.

The levels of serum cytokines TNF, IFN-γ, and IL–10 were shown to be high compared with the non-endemic control, as reported by Correa-Oliveira et al. (23) and Viana et al. (24), and one previous local study among endemic non-infected non-fishermen (25). Among *S. haematobium* endemic area, high level of TNF and low production of IL–10 were observed, which are similar to the finding of Wamachi et al. (26) and King et al. (27).

Conclusion

The biomarkers assayed in this work showed that either there is a possibility that fishermen are exposed to clinically undiagnosed or asymptomatic schistosomiasis. Otherwise those fishermen were exposed to other infectious agents resulting in a higher high levels of cytokines response including parasites. Therefore, further investigation is required to uncover the high levels of other parasites that causes the elevated IgE among fishermen.

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Competing Interests

All authors declared that they have no competing interests.

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