

Humoral and Cytokines Response in Acute Urinary Schistosomiasis Patients in White Nile State (Sudan)

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ABSTRACT

The production and regulation of IFN- γ , IL-2 (Th1 cytokines), IL-4, IL-5 and IL-10 (Th2 cytokines) was evaluated in (93) individuals, (61) with acute schistosomiasis patients and (32) as control group. Humoral response also was evaluated by the different levels of IgM, and IgE in both groups. The objectives of this study was to determine humoral, and cytokines response in acute schistosomiasis in individual living in White Nile State (Sudan) and measurement Th1 cytokines (IFN- γ , and IL2) and Th2 cytokines (IL4, IL5, and IL10) in both acute schistosomiasis patients and control groups. Urine and blood samples were taken from both groups, ten mL of urine were examined for the presence of *S. haematobium* eggs and two mL of whole blood were collected for cytokines and IgE, and IgM determination. The levels of IFN- γ , IL-2, IL-4, IL-5, and IL-10 and circulating level of IgE, and IgM in serum were determined by enzyme-linked immunosorbent assay (ELISA). The study showed that IFN- γ , and IL-2 mean levels to *Schistosoma haematobium* soluble egg antigen (SEA) was significantly higher ($p < 0.05$) in the acutely infected patients (210.80 ± 202.89 , 20.34 ± 39.63), respectively than the control group which were (35.04 ± 68.28 , 3.68 ± 2.78) respectively table (1). The antigen-specific IL-4 responses were also (11.20 ± 12.08) significantly higher ($p < 0.05$) levels in patients group with acute infection compared to control group which were (4.48 ± 6.53) table (1). The means levels of IgM antibody to schistosome-egg antigen (SEA) were (0.38 ± 0.13) significantly higher ($p < 0.05$) in acutely infected patients compared to normal group which were (0.002 ± 0.0001) table (1), in contrast the level of IgE antibody to (SEA) was (436.31 ± 223.31) significantly increased ($p < 0.05$) in acute group compared to control group which were (41.47 ± 39.67) table (1). After Twenty-one days treatment with praziquantel, the treated egg-negative patients were showed significantly decreased ($p < 0.05$) in the production of IFN- γ , IL2, IL-4, and IL5 levels table (2). In conclusion, this study has demonstrated that the pro-inflammatory type 1 cytokines were elicited during acute urinary schistosome infection. And the switch from Th1 to Th2 cytokine dominance has been attributed to the development of the human immune response to the eggs throughout the infection.

Keywords: *Schistosomiasis, Cytokines, Humoral and Acute.*

1. INTRODUCTION

Schistosomiasis or bilharzia is a tropical parasitic disease caused by blood dwelling flukes of the genus *Schistosoma*. It affects about (200) million people while (500) million are at risk of schistosomiasis worldwide. More than (650) million people live in endemic areas (Gryseels et al., 2006; WHO, 2002). Five different schistosome species (*Schistosoma mansoni*, *Schistosoma japonicum*, *Schistosoma haematobium*, *Schistosoma intercalatum*, and *Schistosoma mekongi*) were determined. The geographical distribution of the various *Schistosoma* species depends on the availability of a suitable snail host (Ross et al., 2002). Schistosomiasis is a major parasitic disease in Africa (Hotez et al., 2009). In Sudan, schistosomiasis is the most serious health problem and it is a major cause of morbidity, approximately about (80%) of the Sudanese population at risk, and more than five million are infected by one or more species of the parasite (WHO, 2012). Acute schistosomiasis (Katayama fever) is a systemic hypersensitivity reaction against the migrating schistosomes occurring a few weeks to months after a primary infection (Bottieau et al., 2006; Horak et al., 2005). The disease starts suddenly with fever, fatigue, myalgia, malaise, non-productive cough, eosinophilia, and patchy infiltrates on chest radiography. Abdominal symptoms can develop later, caused by the

migration and positioning of the mature worms. Most patients recover spontaneously after (2—10) weeks, but some develop persistent and more serious disease with weight loss, dyspnoea, diarrhoea, diffuse abdominal pain, toxemia, hepatosplenomegaly and widespread rash. T helper cells (also known as the cells) are a sub-group of lymphocytes, a type of white blood cell that plays an important role in establishing, and maximizing the capabilities of the immune system. The cells are involved in activating and directing other immune cells and are particularly important in the immune system. They are essential in determining B cell antibody class switching in the activation and growth of cytotoxic T cells. This diversity in function and their role in influencing other cells that gives T helper cells their name. Mature The cells are believed to always express the surface protein CD⁴⁺, and are referred to as CD⁴⁺ T cells. More than fifteen interleukins are known and they are designated numerically, IL-1 through IL-15. The immunological functions of most of the interleukins are known to some degree. IL-1, and IL-2 are primarily responsible for activating T and B lymphocytes white blood cells integral to bringing about the acquired immune response, with IL-2 being a stimulant of T, and B-cell growth and maturation. IL-4 often leads to an increase in antibody secretion by B lymphocytes (Bezerra et al., 2007).

2. MATERIALS AND METHODS

2.1 Ethics Statement

Permission to conduct this study in the region was obtained from the Health Services Director-Elduiem locality and village chiefs. Institutional and ethical approval was received from the University of Bacht el.ruda. Only compliant participants were recruited into this study and they were free to drop out at any point during the study. At the beginning of the study participants, and their parents/guardians had the aims, and procedures of the project explained to them. Written informed consent was obtained from the eligible individuals before the starting of the study from 2010 up to 2013.

2.2 Inclusion Criteria

- Exposure to the parasite at the time of initiation of the study (day 0).
- Signing the inform consent.
- Be negative for intestinal helminths including *S. mansoni*, negative for *Plasmodium*. Parasites and other parasitic infections.
- Not have received prior treatment for helminth infections.

2.3 Acute Patients and Control Groups

Sixty- one *Schistosoma haematobium* egg-positive patients with a history of a recent travel to the study area and freshwater contact. The patients were evaluated in their second month of infection and each had been symptomatic for about six weeks. These symptoms consisted of persistent fever, fatigue, cough, abdominal pain and diarrhea (Cline et al., 1977; Katz et al., 1972 and Campi-Azevedo et al., 2008). Thirty two samples were obtained from healthy individuals never been exposed to *S. haematobium* infection and with negative urine examination that served as normal control group.

2.4 Urine and Blood Samples Collection

Urine samples for parasitological examination for *S. haematobium* infection were collected from all the individuals and were processed on the day of collection. Samples were collected between (10:00 a.m. and 2:00 p.m.) to coincide with the peak of *S. haematobium* egg excretion in urine (Warren et al., 1978). Urine samples were examined for urinary schistosomiasis by centrifugation of 10 mL, examined for eggs of *S. haematobium* and counts were expressed as eggs per 10 mL of urine (egg/10 mL- average) (Mott et. al., 1982). Ten mL of urine samples were obtained and examined for the presence of *S. haematobium* eggs from both groups. Blood and urine samples were taken then eggs detection / count were performed at the time of sampling. Serum were collected after centrifugation, stored, and frozen at (-80°C) for cytokines and antibodies measurements, also the *Schistosoma haematobium* positive individuals were monitored for evidence of acute symptoms related to schistosomiasis infection depending on history, and clinical findings. Two mL of whole blood were collected in plane container without anticoagulant. Serum were

collected after centrifugation, aliquoted and stored frozen at (-80°C) for cytokines, and antibodies measurement.

2.5 Circulating cytokines measurements

Levels of IFN- γ , IL-2, IL-4, IL-5, and IL-10 in serum were determined by enzyme-linked immunosorbent assay (ELISA) based prediction kits from eBioscience (San Diego, CA) with specific monoclonal antibody (MAb) pairs. Corning castor 9018 (ELISA) plates were coated with 100 μl /well of captured antibody in coating buffer (diluted as noted on leaflet of analysis, which included with reagent set provided be eBioscience, inc). The (ELISA) test based on the original method of the Engvall and Perlman (1971).

2.6 Human Antibodies Measurement

Circulating levels of IgE, and IgM directed against *Schistosoma haematobium* soluble egg antigen (SEA) purchased from Biological Supply Center (BSC) Egypt (Lot. No 281011), were detected by enzyme-linked immunosorbent assays (ELISA). Wells of polystyrene microtiter plates (corning costar 9018) were coated with 100 μl of *Schistosoma haematobium* (SEA) 30 $\mu\text{g}/\text{mL}$ in coating buffer (carbonate bicarbonate PH 9.6) per well, then incubated overnight at (4°C).

2.7 Statistics Analysis

All data are reported as means \pm SD. Data are analysis was performed using Statistical Package for Social Sciences (SPSS) version 16 USA. Statistical significance was considered as ($p < 0.05$).

3. RESULTS AND DISCUSSION

This study focused on the development of urinary schistosome-specific cytokine responses, with the host clinical form of schistosomiasis acute to determine whether there were differences in the expression of Th1 and Th2 cytokines represented by IFN- γ , and IL-2 as a marker for Th1 cytokines and IL-4, IL-5, and IL-10 as a marker for Th2 cytokines. Also to look for the humoral response by measuring the immunoglobulin M, and E. The results of IgM antibody to schistosome-egg antigen (SEA) was (0.38 ± 0.13) significantly increased ($p < 0.05$) in acutely infected patients of schistosomiasis compared to normal control group which were (0.002 ± 0.0001) table (1), but IgE was (436.31 ± 223.31) higher significantly increase ($p < 0.05$) in acute patient group compared to control group which was (41.47 ± 39.67) table (1). The results of IFN- γ , and IL-2 level were (210.80 ± 202.89 , 20.34 ± 39.63) highly significantly increased ($p < 0.05$) in the acutely infected patients compared to control group which were (35.04 ± 68.28 , 3.68 ± 2.78) respectively table (1). The results obtained were identical with the findings of Chikunguwo et al., (1992); Seder et al., (1993); Abbas et al., 1996 and Takafira, et al., (2009), this may be explained that Gamma interferon (IFN- γ) is a potent activator of macrophages, and other inflammatory mediators during the first three weeks of the infection, when the host is exposed to migrating immature, and mature stages of the parasites. The response is induced by non-egg antigens, such as the cercariae, schistosomula,

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and schistosome worm antigens, (SWA) (Wynn et al., 2004; Gobert et al., 2007; Pearce et al., 2002 and Pearce et al., 2005) or at the beginning of the production of eggs by parasites around four weeks, and this also supported by the reports of Ding et al., (1998) and Pearce et al., (1991). These findings elucidated that Th1-associated cytokines are expressed preferentially at earlier time points, before or in the first few days of egg laying. The results of IL-4, IL5, and IL10 (11.20±12.08, 11.95±14.17, and 34.56±31.64) were significantly ($p < 0.05$) higher in acute patients compared to control group which were (4.48±6.53, 1.44±3.08, and 9.58±15.20) respectively table (1), this findings were observed by Silva et al., (2004); Montenegro et al., (1999); Nguyen et al., (2006), or for that the effect of IL-4 in eliciting the Th1 response is limited to the initial stage of infection (Jones et al., 2002, Wynn et al., 1998). Although many studies have supported a role for IL-10 in the down-regulation of type 1 cytokine responses (Wynn et al., 2004; Gobert et al., 2007), this study have shown that both type 1 and type 2 cytokine responses were at least partially down-regulated at the stages of infection, even in

the similar levels of IL-10 in acute group (34.56±31.64) table (1), similar findings were reported by Pearce et al., (2005). These data strongly suggest that IL-10 may not be the sole mechanism for down-regulating CD4⁺ T cell responses to urinary schistosomiasis. Indeed, Th1 and Th2 cell response shift may preferentially induce by different schistosome antigens during different stages of infection modulated by the immune interaction between hosts and parasites (Montesano et al., 1990). It is not surprising that after Twenty-one days treatment with praziquantel, the treated egg-negative patients were showed significantly decreased ($p < 0.05$) in the production of IFN- γ , IL2, IL-4, and IL5 levels which were (126.50±224.31, 9.92±18.02, 10.94±14.81, and 10.94±14.81) respectively compare to acute infection groups table (2), but not IL10 levels not change in both groups. This can be explained by exposure of released parasite-derived antigens to the immune system following the destruction of worms by treatment. Identical findings were obtained by (Tweyongyere et al., 2008; Martins-Leite et al., 2008).

Table 1: Comparison between levels of cytokines in acute patients and control groups

Parameters	Acute group (n=61)	Control group (n=32)
INF gamma (Pg/mL)	210.80±202.89 ^a	35.04±68.28 ^b
IL2 (Pg/mL)	20.34±39.63 ^c	3.68±2.78 ^d
IL4 (Pg/mL)	11.20±12.08 ^c	4.48±6.53 ^f
IL5 (Pg/mL)	11.95±14.17 ^g	1.44±3.08 ^h
IL10 (Pg/mL)	34.56±31.64 ⁱ	9.58±15.20 ^j
IgM μ L	0.38±0.13 ^k	0.002±0.0001 ^l
IgE μ L	436.31±223.31 ^m	41.47±39.67 ⁿ

Values are means \pm SD. Means with rows not sharing common letter (s) are significantly different ($P < 0.05$).NS= non- significant.

Table 2: Comparison between levels of cytokines in patients before treatment and control groups

Parameters	Acute group (n=61)	Acute before treatment group (n=61)	Control group (n=32)
INF gamma (Pg/mL)	210.80±202.89 ^a	126.50±224.31 ^b	35.04±68.28 ^c
IL2 (Pg/mL)	20.34±39.63 ^d	9.92±18.02 ^e	3.68±2.78 ^f
IL4 (Pg/mL)	11.20±12.08 ^g	10.94±14.81 ^h	4.48±6.53 ⁱ
IL5 (Pg/mL)	11.95±14.17 ^j	10.94±14.81 ^k	1.44±3.08 ^l
IL10 (Pg/mL)	34.56±31.64 ^m	9.12±11.68 ^{iNS}	9.58±15.20 ^{iNS}

Values are means \pm SD. Means with rows not sharing common letter (s) are significantly different ($P < 0.05$).NS= non- significant.

4. CONCLUSION AND RECOMMENDATION

This study has demonstrated that the pro-inflammatory type 1 cytokines were elicited during early urinary schistosome. Additionally, Th1/Th2 regulation and dominance may contribute additional mechanisms that modulate the immune interaction between hosts and parasites. Future studies must continue to improve approaches to study the human immune response to urinary schistosomiasis, and Th1/Th2 regulation, when

this goal is achieved, effective methods of manipulating the immune system, such as vaccination, and treatment can be developed.

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