Humoral and Cytokines Response in Chronic Urinary Schistosomiasis Patients in White Nile State - Sudan

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ABSTRACT
The production and regulation of IFN-γ, and IL-2 (Th1 cytokines), IL-4, IL-5, and IL-10 (Th2 cytokines) were evaluated in (93) urinary Schistosomiasis, egg-positive patients from White Nile State (61) with chronic urinary Schistosomiasis and (32) as control group. Humoral response was also evaluated by the different levels of IgM and IgE in both groups. The objective of this study was to determine Humoral, and cytokines response in chronic 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 chronic Schistosomiasis patients and control groups. Ten mL of urine samples were obtained and examined for the presence of S. haematobium eggs from both groups. Serum were collected after centrifugation, aliquoted and stored frozen at (-80°C) for cytokines, and antibodies measurement. Levels of IFN-γ, IL-2, IL-4, IL-5, and IL-10 in serum were determined by enzyme-linked immunosorbent assay (ELISA). The Circulating levels of IgE, and IgM directed against Schistosoma haematobium soluble egg antigen (SEA). The study showed that IFN-γ was highly significant (p<0.05) in the chronic infected patients group compared to control which were (54.78±66.96, 35.04±68.28) respectively but IL2, IL-4, IL5 showed significant (p<0.05) in the chronic infected patients group compared to control which were (11.31±19.11, 8.97±11.31 and 12.39±26.96) respectively compared to control group which were (3.68±2.78, 4.48±6.53 and 1.44±3.08) Table (1). The mean levels of IgM and IgE were also significantly different between chronic infection patients group compared to control which were (0.18±0.10 and 615.69±990.57) in chronic and were (0.002±0.0001 and 41.47±39.6) in control group Table (1). After twenty-one days treatment with praziquantel the means levels of IFN-γ, IL2, IL4, IL5 and IL10 (54.87±66.96, 11.31±11.11, 8.9f7±11.31, 12.39±26.96 and 34.94±89.26) before treatment were significantly higher compared to the mean level after treatment which were (66.59±105.74, 9.41±19, 65, 8.76±17.89, 8.98±11.79 and 5.27±5.26) were reduced. In both groups before and after treatment the level were high when compared to control group Table (2). In conclusion, this study has demonstrated the pro-inflammatory type 1 cytokines were elicited during chronic urinary Schistosomiasis infection. And also after treatment were increased.

Keywords: Schistosomiasis, Chronic, Humoral Response, Cytokines.

1. INTRODUCTION
Schistosomiasis or bilharzia is a tropical parasitic disease caused by blood dwelling flukes of the genus Schistosoma. It effects 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 Schistosomes species Schistosoma mansoni, Schistosoma Gyrseels japonicum, Schistosoma haematobium, Schistosomes intercalatum, and Schistosoms mekongi were determined. The geographical distribution of the various Schistosomes 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). Achronic Schistosomiasis (Katayama fever) is a systemic hypersensitivity reaction against the migrating schistosomulae occurring a few weeks to months after a primary infection (Botteau  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 T 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 helminthes including S. mansoni, negative for plasmodium parasites and other parasitic infections.
· Not have received prior treatment for helminth infections

2.3 Chronic Patients and Control
Sixty- one Schistosoms haematobium egg-positive patients with chronic Schistosomiasis were selected from a study population that infected for more than one year and none were symptomatic with their chronic infections but they have a sign of terminal hematuria throughout this period assessed by questionnaire (Cline et.al., 1977). Patients in this group assessed by qualified doctors and had never been treated for S. haematobium. 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.

2.4 Treatment of Patients by Praziquantel
All patients with evidence of infection should be treated regardless of symptoms, since adult worms can live for years. Praziquantel is extremely well-tolerated and is used both for treatment of individual patients and in mass community treatment programs.

Praziquantel induces ultra structural changes in the teguments of adult worms, resulting in increased permeability to calcium ions. Calcium ions accumulate in the parasite cytosol, leading to muscular contractions and ultimate paralysis of adult worms. By damaging the tegument membrane, praziquantel also exposes parasite antigens to host immune responses. These effects lead to dislodgement of worms from their intestinal sites and subsequent expulsion by peristalsis. The effectiveness of praziquantel therefore depends at least in part on host immune defenses (Grandière-Pérez, et.al 2006).

2.5 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).

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 chronic symptoms related to Schistosomiasis infection depending on history, and clinical findings. Two mL of whole blood were collected in plate container without anticoagulant. Serum were collected after centrifugation, aliquoted and stored frozen at (~80°C) for cytokines, and antibodies measurement.

2.6 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 eBioscince (San Diego, CA) with specific monoclonal antibody (MAb) pairs. Cornig caster 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 eBioscince, Inc). The (ELISA) test based on the original method of the Engvall and Perlman (1971).

2.7 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 micro titer plates (corning costar 9018) were coated with (100 μL) of Schistosomes haematobium (SEA) (30 μg/mL) in coating buffer (carbonate bicarbonate pH 9.6) per well, then incubated overnight at (4°C).

2.8 Statistics Analysis
All data are reported as means ± 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 Schistosoms-specific cytokine responses, with the host clinical form of Schistosomiasis chronic to determine whether there were differences in the expression of Th1 and Th2 cytokines represented by IFN-γ, and IL-2 as an antibody to Schistosoms-egg antigen (SEA) were significantly increased ($p<0.05$), $54.78\pm66.96, 11.31\pm19.11$ in chronic infected patients of Schistosomiasis compared to normal control group which were $(35.04\pm68.28, 3.68\pm2.78)$ respectively Table (1).

The means levels of IFN-γ and IL-2 were highly significantly ($p<0.05$) in chronic patient compared to control group which were $(54.78\pm66.96, 11.31\pm19.11$ Pg/mL) respectively in chronic and $(35.04\pm68.28, 3.68\pm2.78$Pg/mL) respectively in control group table (1).

Most of chronic patients presented significantly high level of cytokines (IFN-γ, IL5 and IL10) with low production of IL4 indicating that immune response to Schistosomiasis antigen in chronically infected individuals was typically polarized towards Th2 cytokines. This finding also reported by booth et al., 2004 : Abath et al., 2006. The secretion of Th1 cytokines (IFN-γ and IL2) is concurrently down regulated at time when Th2 response begins the transition into the chronic stage of infection (Abath et al., 2006). Similar study support these finding done by Xinyn et al., 2010, observed that during the early stage of infection, Th1-type polarized response and Th2-based apoptosis induced by non-egg antigen. Chronic patients of both groups before and after treatment were showed significantly increased in production of cytokines compared to control groups except IL4 which showed slight increased Table (2). 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 Tweeneyere et al., 2008; Martins-Leites et al., 2008.

| Table 1: Comparison between levels of cytokines in acute patients and control groups |
|-----------------------------------------------|-----------------------------------------------|
| Parameters                                    | chronic group (n=61)                           | Control group (n=32)                           |
| INF gamma (Pg/mL)                             | $54.78\pm66.96^a$                             | $35.04\pm68.28^b$                             |
| IL2 (Pg/mL)                                   | $11.31\pm19.11^c$                             | $3.68\pm2.78^d$                              |
| IL4 (Pg/mL)                                   | $8.97\pm11.31^e$                              | $4.48\pm6.53^f$                              |
| IL5 (Pg/mL)                                   | $12.39\pm26.96^g$                             | $1.44\pm3.08^h$                              |
| IL10 (Pg/mL)                                  | $34.94\pm89.26^i$                             | $9.58\pm15.20^j$                             |
| IgM µL                                        | $0.18\pm0.10^k$                                | $0.002\pm0.0001^l$                           |
| IgE µL                                        | $615.69\pm90.57^m$                            | $41.47\pm39.67^n$                            |

Values are means ±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 chronic patients before and after treatment and control groups |
|---------------------------------------------------------------|-----------------------------------------------|
| Parameters                                    | Chronic before treatment (n=61) | Chronic after treatment (n=61) | Control group (n=32) |
| INF gamma (Pg/mL)                             | $54.78\pm66.96^c$                             | $66.59\pm105.74^b$               | $35.04\pm68.28$      |
| IL2 (Pg/mL)                                   | $11.31\pm19.11^c$                             | $9.41\pm19.65^d$                 | $3.68\pm2.78$       |
| IL4 (Pg/mL)                                   | $8.97\pm11.31^e$                              | $8.76\pm17.89^e$                 | $4.48\pm6.53$       |
| IL5 (Pg/mL)                                   | $12.39\pm26.96^g$                             | $8.96\pm11.79^h$                 | $1.44\pm3.08$       |
| IL10 (Pg/mL)                                  | $34.94\pm89.26^i$                             | $5.27\pm5.69^i$                  | $9.58\pm15.20$      |

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

4. CONCLUSION AND RECOMMENDATION

In conclusion, this study has demonstrated the pro-inflammatory type 1 cytokines, were elicited during chronic urinary Schistosoms infection. And also after treatment were increased.

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