The Effect of Praziquantel Treatment on Humoral and Cytokines Response on both Chronic and 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) were evaluated in -120- urinary schistosomiasis, egg-positive patients from White Nile State -61- subjects with chronic and acute urinary schistosomiasis and -32- subjects as control group. Humoral responses were also evaluated by the different levels of IgM and IgE in both groups of acute and chronic patients. The objective of this study was to determine Humoral, and cytokines response in chronic and acute schistosomiasis individuals living in White Nile State (Sudan), and measurement Th1 cytokines (IFN-γ, and IL2) and Th2 cytokines (IL4, IL5, and IL10) in both chronic and acute schistosomiasis patients and control groups. Ten mL of urine samples were obtained from each subjects and were0 examined for the presence of S.haematobium eggs from both chronic and acute groups. Serum were collected from each subjects after centrifugation, and stored frozen at (−80°C) for cytokines, and antibodies measurement. The 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 were detected against Schistosomahaematobium soluble egg antigen (SEA). The study showed that chronic group level of cytokines were significantly (p< 0.05) lower compared to acute group before treatment , but acute showed significantly (p< 0.005) when compared to both chronic and control groups. IgE presented higher level in chronic group compared to acute and control groups Table (1). The cytokines response in chronic group represented significantly high level of IFN-γ, IL-2, IL-4, IL-5, and IL-10 before and after treatment compared to control group. After treatment there was reduced in the level Table (2). In acute treatment group the level of cytokines were reduced after treatment compared with control group Table (3). In conclusion, after twenty-one days treatment by praziquantel the level of cytokines were reduced in acute group compared to control , but in chronic were higher after treatment, Table 2.

Keywords: Schistosomiasis, chronic, humoral response, cytokines praziquantel.

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 schistosomiaspecies Shistosomamansoni, Schistosoma Gryseels 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). Achronicschistosomiasis (Katayama fever) is a systemic hypersensitivity reaction against the migrating schistosomulae 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, toxaemia, 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.

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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 stimulator 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). Praziquantel is extremely well-tolerated and is used both for treatment of individual patients and in mass community treatment programs.

Praziquantel induced 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. MATERIALS AND METHODS

2.1 Ethics Statement
Permission to conduct this study in the region was obtained from the Health Services Director-Elduieum locality and village chiefs. Institutional and ethical approval was received from the University of Bachtel.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 helmint infections.

2.3 Chronic, Acute Patients and Control
Sixty-one Schistosomahaematobium 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. Sixty-one Schistosomahaematobium 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 Treatment of Patients by Praziquantel
All patients with evidence of infection should be treated regardless of symptoms, since adult worms can live for years. The drugs taken according to recommended standard regimen of 40 mg/kg body weight in a single dose (WHO, 2002).

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 Schistosomahaematobium 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 plane 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. 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 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 Schistosoma 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 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 chronic and acute patients to determine whether there were differences in the expression of Th1 and Th2 cytokines represented by IFN-γ, IL-2, IL-4, IL-5, and IL-10. The means level of IFN-γ, IL-2 and IL-4 in acute patients were increased with significantly (p< 0.05) different which represented (210.80±202.98, 20.34±39.63 and 11.20±12.08 Pg/mL) respectively compared to chronic group which were (54.78±66.96, 11.31±19.11 and 8.97±11.31 Pg/mL) respectively Table (1).

IL-10 means levels in both chronic and acute which were (34.56±31.64 and 34.94±89.26Pg/mL) were presented same results but decreased when compared to control group which (9.58±15.20 Pg/mL) were compared to both group chronic and acute. This finding also reported by Booth et al., 2004; Abath et al., 2006, the secretion of Th1 cytokines (IFN-γ and IL-2) is currently down regulated at time when Th2 response begins the transition into the chronic stage of infection. Similar study support these finding done by Xinyn et al., 2010; observed that during early stage of infection , Th1 type polarized response and Th2- based apoptosis induced by non-egg antigen chronic patients. After treatment the means level of IL-2, IL5 and IL10 were significantly (p< 0.05) reduced which were (9.92±18.02, 8.96±11.79 and 5.27±5.69Pg/mL) respectively compared to the means level before treatment which were (11.31±19.11 ,8.97±11.31 and 34.94±89.26Pg/mL) Table (2).

The means level of INF-γ, IL-2, IL-4, IL-5, and IL-10 in both stage of treatment before and after were significantly higher than control group Table (3). This can be explained by exposure of released parasite-derived antigens to the immune system following the destruction of worms by treatment, identical finding were obtained by Twenty one et al., 2008; Martins et al ., 2008).

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<tr>
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<th>Chronic group (n=61)</th>
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Values are means ±SD. Means with rows not sharing common letter(s) are significantly different (P < 0.05). NS = non-significant.

4. CONCLUSION

After twenty one days treatment by Praziquantel the level of cytokines were reduced in acute group compared to control.

REFERENCES

[18] XinynXu, Xiaoyun Wen, Ying Chi, Lei He,1 Sha Zhou,1 Xuefeng Wang,1 Jiaqing Zhao,1 Feng Liu,1 and Chuan Su (2010) "Activation-Induced T Helper Cell Death Contributes to Th1/Th2 Polarization following Murine Schistosoma japonicum Infection (2010) . " Journal of Biomedicine and Biotechnology Volume Article ID 202397, 12 pagesdoi:10.1155/2010/202397