DETECTION OF CIRCULATING ANTIGENS IN PATIENTS WITH ACTIVE SCHISTOSOMA HAEMATOBIUM INFECTION

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Abstract. An antigen-capture ELISA using monoclonal antibody (MAb) 128C3/3/21 was used to detect circulating parasite-derived antigens in the sera of patients actively infected with Schistosoma haematobium (31 males and four females, 5–25 years of age). The assay had a sensitivity of 100% (35 of 35 patients with antigen levels > 80 ng/ml) and a specificity > 99%. We used this ELISA to monitor antigenemia before treatment and one, three, and six months after treatment with a single oral dose of praziquantel (PZQ) (60 mg/kg in 20 patients or 40 mg/kg in 15 patients) and compared our findings with those indicated by other measures of disease progression. Circulating antigen levels decreased drastically after PZQ treatment (P < 0.001), with a significantly higher decrease occurring after treatment with 60 mg/kg than with 40 mg/kg. Although the mean antigen level was still significantly reduced (P < 0.001) at six months after treatment, 16 patients remained antigen-positive after six months, and nine had increased levels of antigenemia, reflecting reinfection in six patients and persistence of infection in another. We observed a correlation (r = 0.6, P < 0.01) between the level of circulating antigen and the intensity of infection as measured by egg count. We also found a direct relationship (P < 0.001) between antigen level and the severity of the disease as monitored by ultrasonography. We conclude that our ELISA may be a useful adjunct to other methods, such as ultrasonography, for monitoring the course of S. haematobium infection and treatment.

Schistosomiasis is second only to malaria in terms of its worldwide spread, affecting about 200 million people in 75 countries.1 Schistosoma haematobium remains the most prevalent cause of schistosomiasis in upper and middle Egypt.2 Over the last 10 years significant progress has been achieved in the diagnosis of schistosomiasis, and steadily increasing efforts have been directed toward the long-term goal of producing an anti-schistosome vaccine.

We have previously developed1 and refined (Hassan M, Hammad T, unpublished data) a monoclonal antibody (MAb)–based antigen-capture ELISA to detect parasite-specific antigens in the serum of individuals infected with S. mansoni. This ELISA uses an MAb designated 128C3/3/21, which recognizes an unusual carbohydrate epitope that is expressed at all stages of parasite development.5, 6 In its original form,7 the assay had an overall sensitivity of 78%, with a sensitivity of 100% for individuals excreting > 100 eggs/g of feces and 72% for those excreting < 100 eggs/g of feces. It showed a specificity > 99% for sera from age-matched, uninfected controls and from age-matched individuals infected with parasites other than Schistosoma. Although this assay showed a clear correlation between the level of circulating schistosome antigens and the intensity of infection (as indicated by egg counts), we did not find any relationship between the extent of the antigenemia and the clinical stage of the disease as determined by traditional staging criteria.3 The same lack of correlation between circulating parasite-derived antigen and clinical stage was observed in patients infected with S. japonicum.8 These findings suggest that the clinical stage, as traditionally assessed, may not be a good reflector of the actual morbidity produced by the disease and that alternate approaches such as ultrasonography9 and quantitation of antigenemia may also be useful in monitoring disease progression.

Elevation of serum IgE, IgM, and IgG levels in schistosomiasis is well documented.9 As in other helminthic infections, this antibody response seems to be the result of polyclonal B cell activation, in which parasite-specific antibodies constitute only a minor portion of all the immunoglobulins produced.10 Most of the antibodies begin to appear in appreciable quantities at the time of parasite maturation and ovisposition in the tissues.11, 12 One method of quantitating antibody production in schistosomiasis haematobium has been to measure the levels of anti-soluble adult S. haematobium worm antigen preparation (SWAP) in infected patients before and after treatment.13

The aims of the present study were 1) to evaluate the ability of our ELISA to detect the presence of parasite-specific antigens in the serum of patients infected with S. haematobium and 2) to compare the degree of antigenemia indicated by our assay to the level of morbidity demonstrated by other indicators of disease progression (egg count, ultrasonography, and ELISA of circulating anti-SWAP IgG1, IgG4, and IgE) in these patients before and after treatment with praziquantel (PZQ).

PATIENTS, MATERIALS, AND METHODS

Patients. Thirty-five patients infected with S. haematobium (31 males and four females, 5–25 years of age [mean ± SD = 13.9 ± 4.4]) were randomly selected from the outpatient clinic of the Tropical Medicine Department, Assiut University Hospital, Assiut, Egypt. All patients were excreting viable S. haematobium eggs in their urine samples. Those concomitantly infected with S. mansoni or other parasites were excluded. All patients were treated with a single dose of PZQ (600 mg/tablet, Distocid; Epico, Cairo, Egypt) and followed-up at one, three, and six months after treatment. Fifteen patients received a single oral dose of 40 mg/kg, and 20 patients received a single oral dose of 60 mg/kg.

Ten age-matched patients from the same geographic area who had parasite infections other than Schistosoma were in-
cluded as an infected control group (three with *Hymenolepis nana*, one with *Enterobius vermicularis*, one with *Fasciola hepatica*, two with *Strongyloides stercoralis*, and three with *Ascaris lumbricoides*). Another 10 age-matched individuals, apparently healthy and showing no evidence of parasitic infection, served as a control group.

Informed consent was obtained from adult patients and parents of children before the study was initiated. The study was reviewed and approved by the Schistosomiasis Research Project Ethical Committee for the Protection of Human Subjects in Medical Research.

**Materials and methods.** Full clinical histories were obtained from all patients, with special emphasis given to symptoms attributable to *S. haematobium* infection or its complications. Complete general and systemic examinations were performed, with particular attention paid to the size and tenderness of the liver, spleen, and kidneys.

All patients were evaluated by abdominal ultrasonographic examination before PZQ treatment and at one, three, and six months thereafter. Morbidity categories defined by Jawier and others were used for this analysis. Liver, portal vein, spleen, splenic vein, kidney, and urinary bladder were screened and the wall of a full bladder was examined for thickness, irregularity, localized hypertrophy, polyps, masses, and calcification. A bladder wall was considered abnormal if it was more than 3 mm thick.

**Urine and stool examination.** Egg counts in 10 ml of urine were obtained for all patients before and at 1, 3, and 6 months after PZQ treatment. The Nucleopore filtration technique (Costar, Cambridge, MA) was used for egg counting: 1–49 eggs/10 ml of urine were considered to indicate a mild infection, 50–99 eggs/10 ml a moderate infection, and ≥100 eggs/10 ml a heavy infection.

Stool examinations according to the modified Kato thick smear method were performed on three consecutive days to detect infection with *S. mansoni* or other parasites.

**Antigen-capture ELISA.** The ELISA to detect circulating schistosome antigen was carried out as previously described and modified (Hassan M, Hammad T, unpublished data) on sera collected from all controls and from *S. haematobium*-infected patients before treatment and one, three, and six months after PZQ treatment.

**Anti-SWAP immunoglobulin assays.** Levels of anti-SWAP IgG1, IgG2, and IgE in patient sera before PZQ treatment and one, three, and six months after treatment were measured by an ELISA technique.

**Statistical methods.** Descriptive statistical analyses were performed. Student’s *t*-test and Pearson’s correlation coefficient (*r*) were used. *P* values were considered significant when they were < 0.05.

**RESULTS**

**Clinical and ultrasonographic findings in *S. haematobium*-infected patients before and after PZQ treatment.** Most of the *S. haematobium*-infected participants in this study (31 of 35 patients) were males (89%) 5–25 years of age, with 22 (63%) of 35 less than 14 years of age. Twenty patients (80%) were found to be mildly infected, i.e., they had 1–49 *S. haematobium* eggs/10 ml of urine, four (11.4%) had a moderate infection (50–99 eggs/10 ml of urine), and only three (8.6%) had a heavy infection.

Egg counts decreased drastically after PZQ treatment at either a dose of 40 mg/kg or 60 mg/kg: mean ± SD = 46.1 ± 73 eggs/10 ml of urine before treatment, 2.9 ± 6.5 eggs/10 ml one month after treatment, 0.34 ± 1.4 eggs/10 ml three months after treatment, and 1.86 ± 7.5 eggs/10 ml six months after treatment. However, six months after treatment, six patients were still passing viable eggs in their urine samples, presumably because of reinfection. Moreover, one patient had viable *S. haematobium* eggs throughout the post-treatment period, indicating failure of the treatment.

Treatment with PZQ also produced a marked decrease in clinical symptoms related to parasitic infection (Table 1). The incidence of dysuria and hematuria decreased dramatically during the first three months after treatment, with a return of symptoms in several patients at six months (suggesting reinfection). Ultrasonographically detected changes in urinary bladder wall morphology as well as kidney back pressure signs responded well to PZQ treatment. No signs were seen at six months, except for a localized bladder wall thickening in one patient.

**Levels of circulating schistosomal antigens in patients before and after PZQ treatment.** In our ELISA, the mean antigen level ± 3 SD for sera from the healthy control group was 80 ng/ml (established as the cut-off value for the assay). All 35 patients with active *S. haematobium* infections had circulating antigen levels > 80 ng/ml, indicating a test sensitivity of 100%. All members of the infected control group had antigen levels < 80 ng/ml, indicating a test specificity > 99%.

The mean levels of circulating antigen decreased drastically (by almost four-fold) after PZQ treatment in the 26 infected patients who were completely cured (i.e., those who had no eggs in their urine samples) (*P* < 0.001; Figure 1A). In 19 of these patients the mean ± SD antigen level before treatment was 336.7 ± 118 ng/ml and all 19 were ELISA-negative after treatment; the mean antigen level in the remaining seven patients was higher before treatment (416 ± 112 ng/ml), and although the antigen level decreased after treatment in these patients, it did not decrease below the cut-off value of 80 ng/ml. The nine patients who were not cured

**Table 1** Improvement in clinical symptoms in 35 *Schistosoma haematobium*-infected patients after praziquantel (PZQ) treatment

<table>
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<tr>
<th>Symptom</th>
<th>No. of patients</th>
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<td></td>
<td>Before PZQ</td>
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<tr>
<td>Dysuria</td>
<td>23</td>
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<tr>
<td>Hematuria</td>
<td>31</td>
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<tr>
<td>Urinary bladder changes</td>
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<tr>
<td>Thick wall</td>
<td>10</td>
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<tr>
<td>Irregular wall</td>
<td>16</td>
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<td>Local thickening</td>
<td>11</td>
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<td>Polyps</td>
<td>3</td>
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<td>Back pressure changes</td>
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<td>Right kidney</td>
<td>2</td>
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<td>Left kidney</td>
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FIGURE 1. A, relationship between mean parasite-specific circulating antigen level in *Schistosoma haematobium*-infected patients and mean egg count in urine samples before and after praziquantel (PZQ) treatment in the 26 completely cured patients. Serum antigen levels were determined by ELISA and egg counts were determined by the Nucleopore filtration method, as described in the Patients, Materials, and Methods. Values are the mean ± SE. B, relationship between mean circulating antigen level and mean egg count before and after PZQ treatment in nine noncured patients. Values are mean ± SE.

(i.e., who were still passing eggs six months after treatment) had an increased antigen level after an initial decrease in antigen concentration (Figure 1B). The drug effect was more pronounced in the patients receiving 60 mg/kg of PZQ than in those receiving 40 mg/kg (*P* < 0.001; Table 2). Furthermore, six months after treatment, the mean ± SD antigen level of the children who were 14 years of age or younger was significantly higher (177.8 ± 108 ng/ml) than that of the older patients (118 ± 103 ng/ml) (*P* < 0.05).

We observed a direct positive correlation between the level of circulating antigen and the egg count before treatment (*r* = 0.6, *P* < 0.01). The mean circulating schistosomal antigen level was also directly related to the progression of urinary bladder morbidity, as evaluated by ultrasonography (*P* < 0.001) (Figure 2).

Levels of circulating anti-SWAP antibodies in patients before and after PZQ treatment. All patients had high levels of anti-SWAP-specific IgG1 and IgG4; however, five of the 35 patients had no detectable anti-SWAP IgE. Treatment with PZQ led to a long-term decrease in all three anti-SWAP
isotypes (Table 3). Whereas the mean IgE level was significantly decreased three and six months after treatment, the mean IgG1 and IgG4 levels had decreased only slightly three months after treatment and were significantly different from pretreatment levels only six months after treatment.

Positive correlations were observed between the levels of IgG1 (r = 0.3698, P < 0.05) and IgG4 (r = 0.3582, P < 0.05) and egg counts. However, no correlation was seen between the level of IgE and egg count (r = 0.1608, P > 0.05).

A significant positive correlation between circulating antigen level and anti-SWAP IgE level was obtained in patients only before treatment with PZQ (r = 0.4547, P < 0.01). No correlation was observed between either IgG1 or IgG4 and antigen level either before or after PZQ treatment.

**DISCUSSION**

We have used an indirect ELISA for the detection of circulating schistosomal antigen in patients infected with *S. haematobium*. Using MAB 128C3/3/21 for antigen capture and biotinylated MAB 128C3/3/21 for detection, we have achieved an overall sensitivity of 100% for 35 patients with parasitologically proven schistosomiasis and a specificity ≥ 99% for 20 individuals free of *S. haematobium* infection.

Our data showed a direct relationship between the mean circulating antigen level and the egg count obtained in infected patients before treatment with PZQ. A similar correlation between circulating antigen levels and egg counts has been observed in *S. mansoni*-infected patients.3, 17 The level of circulating antigen therefore appears to be a good reflector of the intensity of infection, and this ability to measure antigenemia may be particularly useful in epidemiologic studies.

The 35 actively infected patients were mostly male (88.6%) and 14 years of age or younger (62.9%). This distribution is consistent with those in previous studies conducted in Upper Egypt.18, 19 It is also not surprising because women in Upper Egypt generally have a lower degree of exposure to contaminated water than do their male counterparts as a result of differences in the working patterns of the two sexes. Moreover, younger children are more often in contact with water than are members of other age groups.
Most of our patients (80%) also had a mild intensity of infection (passing 1–49 ova/10 ml of urine).

Our study has demonstrated the effect of PZQ treatment in terms of changes in clinical findings (egg count and bladder morbidity), anti-SWAP antibody production, and antigenemia (ELISA results). We observed a significant reduction in both hematuria and dysuria in response to PZQ treatment. Furthermore, we noted a significant regression in bladder morbidity as revealed by ultrasonography six months after treatment. This decrease in morbidity is in agreement with the findings of other investigators,20–22 who have reported that treatment with PZQ reverses the progression of schistosomal granulomatous lesions in the bladder. In addition, the improvement in early back pressure changes that we observed after treatment in the kidneys of our patients has also been reported.20, 22, 23

The urinary bladder is the most severely affected organ in S. haematobium infection.24 In our study, we found that the greater the severity of bladder morbidity (as monitored by abdominal ultrasonography), the higher the mean circulating antigen level (as measured by ELISA). Therefore, high levels of antigen may be indicative of morbidity-related changes in the urinary bladder. The urinary bladder changes result from granuloma formation, which is dependent upon the induction of CD4+ T helper cells (TH cells). Since a reciprocal relationship often occurs between humoral and cell-mediated immunity,25, 26 a relatively decreased level of humoral immunity in patients with severe urinary bladder changes might account for the high level of antigen that we measured in these patients.

As was observed in the present study, a significant decrease in parasite-specific antigen concentration after PZQ therapy has been reported.3, 17, 27, 28 However, despite the fact that the mean level of circulating antigen in our patients decreased after PZQ treatment, 16 patients were still seropositive and nine showed increased levels of antigen six months after treatment. Reinfection in six of these patients was associated with an increase in circulating antigen levels after an initial decrease at one and three months. Furthermore, failure of treatment in one patient was associated with persistence of a high circulating antigen level. High levels of circulating antigen were found in two patients despite negative urine examination results; this apparent contradiction could be explained by day-to-day fluctuations in egg excretion, extensive schistosomal fibrosis that limits egg passage, or differences in the immune status of these hosts. The antigen levels in seven of the 16 patients had decreased but had not reached the cut-off level of 80 ng/ml six months after treatment. This persistent antigenemia could be explained by higher mean ± SD antigen levels among these patients before treatment (416 ± 112 ng/ml versus 336.7 ± 118 ng/ml; P < 0.05), which require a longer period of time to be cleared from the circulation, and/or by differences in the patients’ immune status.

A PZQ dose of 60 mg/kg appeared to give better results than a 40 mg/kg dose based on the data obtained by monitoring the circulating antigen level. Those patients who received 60 mg/kg had significantly lower mean antigen levels six months after treatment than those receiving 40 mg/kg. Analogous results have been reported in S. mansoni-infected patients.28

Although PZQ treatment had a measurable effect on production of anti-SWAP antibodies, the mean levels of anti-SWAP IgG1 and IgG4 in treated patients were found to decrease significantly only six months after treatment. In contrast, the mean level of anti-SWAP IgE decreased more rapidly, i.e., three months after treatment. Shaker and others29 have reported a significant decrease in the level of anti-SWAP IgE two months after treatment in school children infected with S. mansoni, with specific IgG decreasing significantly three months after treatment. A study of Sudanese children infected with mixed S. mansoni and S. haematobium30 reported a remarkable decrease in parasite-specific IgG and IgE antibodies three months after treatment; these investigators advised that a longer follow-up period is needed. IgG4 in S. mansoni-infected Egyptian patients decreased significantly three months after treatment,31 whereas IgG showed no significant decrease at that time. The difference between our results and those of others may be related to differences in the parasite species being examined.

Our study observed a significant positive correlation between the intensity of infection (i.e., egg count) and the levels of anti-SWAP IgG1 and IgG4 but not of anti-SWAP IgE. The magnitude of IgG and IgE responses is related to the intensity of infection.32, 33 In contrast, no correlation between the levels of specific anti-schistosomal IgG4, IgE, and IgM and intensity of infection was found.34 Later studies35, 36 suggested that the level of anti-egg IgG4 relates most closely to the intensity of infection.

We also found a positive correlation between the level of circulating antigen and that of anti-SWAP IgE during active infection. It has been reported that IgE antibodies recognizing adult worm antigens of S. mansoni and S. haematobium clearly increase with parasite load.31 The presence of living parasites is a requirement for IgE production.36 Production of IgE is also dependent on the cytokine interleukin-4, which is secreted by T helper (Th2) cells. IgE has an evident role in immune protection, particularly in antibody-dependent cellular cytotoxicity.37 The high level of IgG4 in active infection supports the hypothesis that a slow build-up of IgE to high levels and an early production of IgG4 antibodies, which may block the IgE pathway,38 are responsible for delaying the development of protective immunity against S. haematobium infection.39, 40 Furthermore, the IgG4 may block mast cell degranulation by competing with specific anti-parasite IgE for the allergic worm antigens.38

Although we detected a high titer of anti-SWAP IgG2 and IgG4 in all 35 patients with active S. haematobium infection, five of these patients showed an extremely low titer of IgE. Theoretically, circulating antigen would be detectable before changes in antibody level and would therefore be more an appropriate target for assays to determine prevalence in endemic areas.41 The presence of antigen in all of our patients suggests the possibility that detection of circulating antigen would be more sensitive than antibody detection in diagnosing active S. haematobium infection.

In a similar vein, it was suggested that antibody assays do not reflect the success or failure of treatment, whereas antigen assays do, since antigens should be rapidly cleared from the host after elimination of the worms.42 In accordance with this suggestion is our observation of a relatively earlier decrease in mean antigen level than in antibody level after
PZQ therapy. Furthermore, in our study the measurement of antigen level was more sensitive than quantitation of antibody levels in detecting reinfection or persistence of infection. In contrast to the result obtained in our ELISA, only one of five patients who were reinfected (as indicated by egg counts) showed an increased IgG level, and only two had increased IgG levels six months after treatment.

In conclusion, we have demonstrated that an MAAb-based antigen-capture ELISA for the detection of parasite-specific antigen is highly sensitive and specific in diagnosing active *S. haematobium* infection and provides valuable data for use in monitoring the efficacy of treatment, reinfection, and persistence of infection. Although this study was preliminary in nature and was conducted on a limited number of patients, the data suggest that further evaluation with large numbers of patients and more prolonged follow-up is warranted to confirm the usefulness of our ELISA as an additional tool for monitoring *S. haematobium* infection and treatment.

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