Kidney International, Vol. 36 (1989), pp. 904–907

A prospective, randomized therapeutic trial for schistosomal specific nephropathy

MOHAMED A. SOBH, FATMA E. MOUSTAFA, SAMIR M. SALLY, MOHAMED ASHRAF M. FODA, ANDRE M. DEELDER, and MOHAMED A. GHONEIM

Urology and Nephrology Center, University of Mansoura, Egypt, and Laboratory of Parasitology, University of Leiden, Leiden, The Netherlands

A prospective, randomized therapeutic trial for schistosomal specific nephropathy. In this work 26 patients with schistosomal specific nephropathy were randomly distributed among three groups. Group I cases were given anti-schistosomal drugs (oxamniquine and praziquantel), group II cases were given anti-schistosomal drugs plus prednisolone, and group III cases were given anti-schistosomal drugs plus cyclosporine. The schistosomal specificity of kidney lesions was assessed by detecting the schistosomal specific antigens (CAA and CCA) and antibodies deposited in the renal glomeruli of these patients. Patients who had another etiologic cause which may explain their kidney disease were not admitted to this study. After initiation of the treatment, patients were followed up every other week in the outpatient clinic for 12 months. Follow-up showed complete remission of proteinuria in two cases in group II (duration of remission was 4 and 8 months) and in one case in group III (duration of remission was 6 months) but in none in group I. Partial remission was observed in one case in group I, in three cases in group II and in one case in group III. During the observation period, improvement in kidney function was observed in two cases in group II but deterioration in kidney function was observed in one case in group I and in one other case in group III. We conclude that in patients with schistosomal nephropathy, none of the tried therapeutic regimens produce regression of the disease if given to patients with established disease.

We have previously reported the existence of schistosomal specific nephropathy in clinical settings and that this disease may progress to end-stage renal failure [1, 2]. The treatment of schistosomal nephropathy is still unknown. The objective of this work was to study the effect of three therapeutic modalities on schistosomal nephropathy.

Patients with active *Schistosoma mansoni* infection with renal involvement by specific lesions were selected for this study. Activity of *Schistosomal* infection was documented by the demonstration of living *Schistosoma mansoni* eggs in the stool or in the rectal mucosal biopsy. Schistosomal specificity of kidney lesion was considered when there was no other cause to explain the kidney disease combined with the demonstration of schistosomal circulating anodic antigen (CAA) and/or circulating cathodic antigen (CCA).

Received for publication November 2, 1988
and in revised form June 6, 1989
Accepted for publication June 15, 1989

Methods

Clinical and laboratory evaluations

Patients with nephropathy and active *Schistosoma mansoni* infection were subjected to evaluations which included thorough history taking and clinical examination, evaluation of the degree of edema, blood pressure, presence of concomitant infection, 24-hour urinary protein, serum creatinine, creatinine clearance, plasma albumin, plasma total proteins, serum cholesterol and complement components C_3 and C_4, L.E. cells, anti-DNA, C-reactive protein, anti-streptolysin-O titer, fasting and post-prandial blood suger.

Kidney biopsy

Two cores of kidney tissue were obtained from the lower pole of the right kidney by percutaneous needle approach.

1. One was fixed in 10% formalin and processed as paraffin sections which were stained with hematoxylin and eosin, periodic acid schiff, mathon trichrome, silver methenamine and Congo red stains, and examined by light microscopy for determination of the pathologic nature of kidney lesions.
2. The second core was immediately deep frozen (−70°C); for immunofluorescent study.

Direct immunofluorescence. Kidney sections were stained by fluorescein-labeled anti-human IgG, IgM, IgA, C_3, C_4, C_{1q} and fibrinogen at dilutions of 1/8 to 1/10 and examined according to the standard procedures.

Indirect immunofluorescence. Frozen sections were examined by using monoclonal anti-schistosomal CAA and monoclonal anti-CCA in a working dilution (1:1000). FITC-labelled anti-mouse IgG was used as a second layer in a working dilution (1:10). These antibodies were prepared as described by Deelder et al [3, 4]. Sections from adult *Schistosoma mansoni* worm fixed in Rossman's fixative were included as positive control, and frozen kidney sections from non-schistosomal cases with idiopathic nephrotic syndrome served as negative control.

Specificity studies. Cases positive for schistosomal antigens by indirect immunofluorescent studies were further subjected to specificity studies, these included the following:

(a) Absorption experiments. The non-labelled monoclonal antibodies (anti-CAA and anti-CCA) with working dilution 1:1000 were incubated at 4°C overnight with adult worm sec-

tions. Kidney sections were re-examined by indirect immunofluorescence using the absorbed antisera as first layer and FITC-labelled anti-mouse IgG as a second layer in a working dilution of 1:10.

b) Tissue elusion study. Only positive cases with sufficient kidney tissue as well as five control cases were subjected to this study. Tissue elusion was performed as previously described [1, 2]. Eluates of schistosoma-positive and control cases were tested against paraffin sections of adult *Schistosoma mansoni* worm fixed in Rossmans fixative. An indirect immunofluorescence test was performed in which the eluate worked as a first layer and FITC-labelled polyvalent anti-human immunoglobulin as second layer.

Therapeutic protocols

Patients with schistosomal specific lesions were randomly distributed among three groups. Group I cases were given anti-schistosomal treatment (Oxamniquine and Praziquantel), group II cases were given anti-schistosomal treatment and prednisolone, and group III cases were given anti-schistosomal treatment and cyclosporine. Oxamniquine was given in a total oral dose of 40 to 60 mg/kg administered on three successive days, and praziquantel was given as a single oral dose of 15 mg/kg.

Prednisolone was given in a dose of 60 mg/day orally for eight weeks, then gradually withdrawn over 24 weeks.

Cyclosporine was given for 12 weeks orally in a dose of 5 mg/kg/day; this was given in two divided doses and readjusted so as to have a whole blood trough level of 200 to 300 ng/ml as measured by polyvalent RIA kits (Sandoz).

Follow-up

Patients were followed up every other week in the out-patient clinic where they were examined clinically and subjected to blood analysis for their serum creatinine, plasma total protein, plasma albumin, serum cholesterol and urine analysis for proteinuria.

End point

End-point for this study was 12 months following initiation of the treatment. At that time patients were evaluated clinically and biochemically as well as by kidney biopsy.

Histopathologic re-evaluation

Patients were readmitted. Cure from *Schistosoma mansoni* infection was assured by absence of living *Schistosoma mansoni* ova in the rectal mucosal biopsy. Kidney biopsy was then performed and was examined as previously described. When microscopic examination by indirect immunofluorescence showed a decrease or disappearance of the schistosomal-specific antigen(s) (CAA and/or CCA) deposits, tissue elution was performed. Sections subjected to tissues elution were re-examined by indirect immunofluorescence using anti-CAA and anti-CCA. Furthermore, eluates of these cases were tested for presence of schistosomal antibodies by using adult worm cross-sections fixed in Rossman's fixative.

Statistical analysis

The results of the laboratory data were expressed as mean ± standard deviation. Statistical analysis was undertaken by Student's paired *t*-test. Significance of differences was accepted at $P < 0.05$.

Table 1. Evaluation of patients subjected to the study

Group	I	II	III
Number	8	10	8
Age	20.2 ±	19.6 ±	22.8 ±
Sex			
M	7	8	6
F	1	2	2
Presentation			
Nephrotic syndrome	7	10	6
Non-nephrotic proteinuria	1	0	2
Hypertension	0	0	0
High serum creatinine	1	2	1
Histopathology			
Mesangio capillary glomerulonephritis	2	4	2
Mesangial proliferative glomerulonephritis	2	3	3
Focal and segmental glomerulosclerosis	3	1	1
Membranous glomerulonephritis	1	1	1
No change	0	1	1

Table 2. Response to treatment

Group	I	II	III
Remission of proteinuria			
Complete	0	2	1
Partial	1	3	1
No	7	5	6
Serum creatinine			
Increase	1	0	1
Stable	7	8	7
Decrease	0	2	0
Histopathologic			
Progression	1	2	1
Stable	7	8	7
Regression	0	0	0
Drug toxicity	0	2	2

Results

Twenty-six patients fulfilled the criteria for admission to the study.

Table 1 shows the clinical, the laboratory and the histopathologic criteria of these patients. Mesangial proliferative glomerulonephritis, mesangiocapillary glomerulonephritis and focal segmental glomerulosclerosis were the common lesions encountered in this group of patients. Most of the patients were nephrotic, normotensive and with normal serum creatinine.

Table 2 shows the response to treatment in the three groups. Following treatment, complete clinical and laboratory remission was documented in two cases in group II and in one case in group III; the duration of remission was four to eight months. Partial remission defined as ±50% reduction in the degree of 24 hour proteinuria was observed in one case in group I, in three cases in group II and in one case in group III. The duration of these remissions ranged from four to eight months also. There was no response to treatment in seven cases in group I, in five cases in group II and in six cases in group III. Cyclosporine was stopped in one case due to acute nephrotoxicity.

Histopathologic re-evaluation showed no regression in lesions in any of the three groups, and progression of the kidney disease was observed in one case in group I, in two cases in

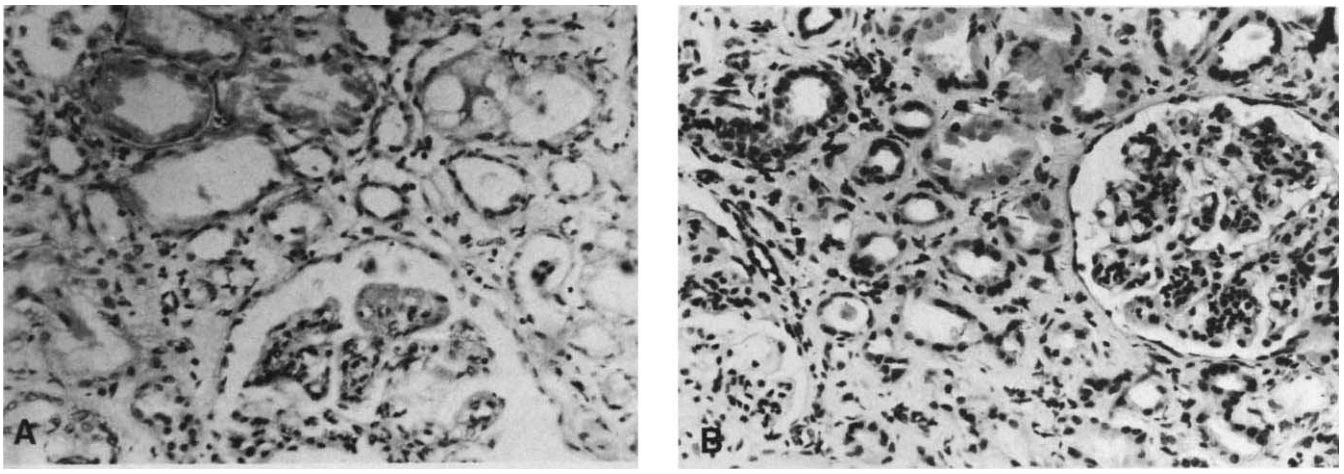

Fig. 1A. *Kidney biopsy of a case from group II before treatment showing segmental hyaline thickening of the basement membrane with mild increase in mesangial cells and interstitial fibrosis (H & E × 250).* **B.** Biopsy from the same patient, after treatment showing advanced mesangial hypercellularity and an increase in the interstitial fibrosis.

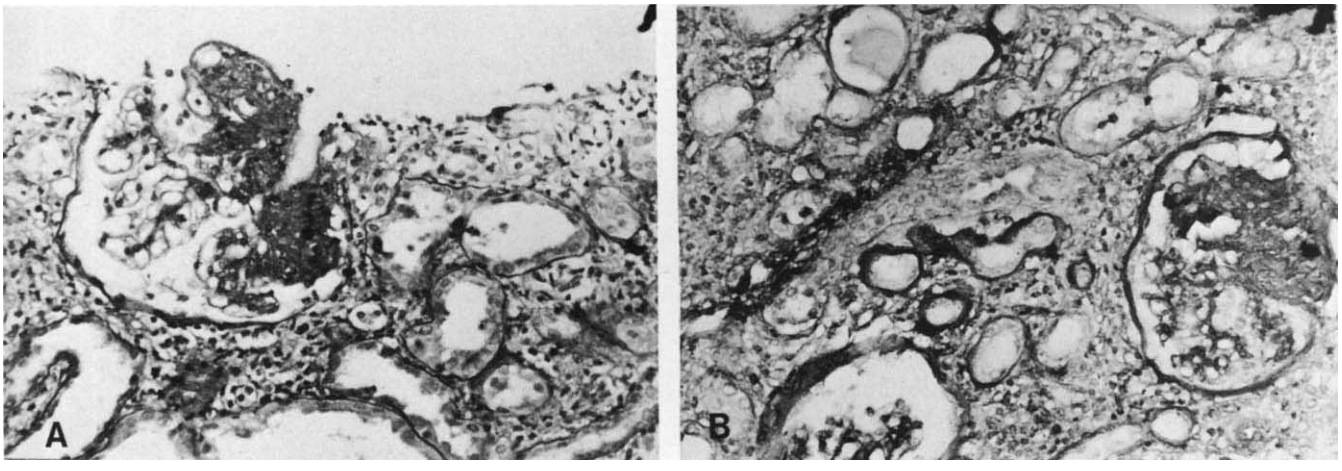

Fig. 2A. *Kidney biopsy of a case from group III before treatment showing focal segmental glomerulosclerosis (PAS × 250).* **B.** Biopsy from the same patient but after treatment showing interstitial fibrosis and atrophic dilated tubules. (PAS × 250).

Table 3. Laboratory evaluation of patients with schistosomal specific nephropathy groups before and after treatment

	I		II		III	
	Before	After	Before	After	Before	After
Serum creatinine	0.82 ± .20	0.85 ± 0.32	0.99 ± 0.45	0.95 ± 0.48	0.68 ± 0.22	0.54 ± 0.18
Plasma total protein	5.8 ± 0.8	5.7 ± 0.7	5.0 ± 0.44	5.8 ± 0.88[a]	5.54 ± 0.91	5.52 ± 0.61
Plasma albumin	2.7 ± 0.8	2.5 ± 0.7	2.3 ± 0.39	2.93 ± 0.4[a]	2.62 ± 0.75	2.6 ± 0.7
Serum cholesterol	380 ± 120	390.8 ± 186	340.7 ± 124.9	278.7 ± 108.9	357 ± 188	337 ± 159
24 Hour proteinuria	3.9 ± 2.1	3.99 ± 2.2	4.47 ± 2.2	3.92 ± 3.47	2.92 ± 1.6	3.56 ± 2.42

[a] $P < 0.01$

group II and in one case in group III. This progression was mainly in form of an increase in the interstitial fibrosis, tubular atrophy and in the extent of the mesangial proliferation (Figs. 1, 2).

Table 3 shows the biochemical evaluations of the patients before initiation of treatment and at the end point of the study. There was no observed statistical difference in group I and III, yet there was significant increase ($P < 0.01$) in the plasma albumin and total protein in group II cases. In addition, there was reduction in the 24 hour proteinuria, but this did not rank to significance ($P > 0.05$).

In no case was there toxicity related to the intake of the anti-schistosomal drugs. In those received prednisolone, one developed osteoporosis and another suffered from steroid-related diabetes and gastrointestinal hemorrhage. In those who received cyclosporine, one suffered from nephrotoxicity and another suffered from hepatotoxicity; these were completely reversible on drug withdrawal.

Discussion

Schistosomiasis is a helmenthic disease of man and animal. A glomerulopathy related to Schistosoma mansoni infection has been described [1, 2, 5, 6]. The presence of schistosomal antigens together with immunoglobulins and complement in the glomerular lesions associated with schistosomiasis has been considered as good evidence of an immune complex mechanism for schistosomal glomerulopathy [7, 8]. Furthermore, Hillyer [9] suggested that DNA and DNA antibodies may play a role in the pathogenesis of renal lesions in schistosomiasis. Thus, schistosomal glomerulopathy resembles that of systemic lupus erythematosus where pathogenesis is mediated by antibodies to DNA. No treatment has been known for schistosomal nephropathy until now. The objective of this work was to study the effects of three therapeutic modalities on schistosomal nephropathy. In all of the three groups, eradication of the parasite by anti-schistosomal drugs was achieved. A combination of two effective drugs was used [10–12]. Absence of living *Schistosoma mansoni* eggs in stool analysis and rectal mucosal biopsies performed 12 weeks after giving the drugs and at the end point of the study confirmed the efficacy of anti-schistosomal treatment and excluded the possibility of re-infection. It is reported that parasitological methods which detect ova are currently superior to serological or other indirect tests of infection [13, 14]. In the second group prednisoline was given as well and cyclosporine was given for those in group three. All patients subjected to this study were of young age and all except three were presenting with nephrotic syndrome. All patients were normotensive, four cases showed mild to moderate elevation in serum creatinine. Mesangial proliferative glomerulonephritis and mesangiocapillary glomerulonephritis were the two most common lesions encountered in these cases. Schistosomal specificity of these kidney lesions was confirmed by absence of other etiologic cause and the demonstration of schistosomal specific antigens and antibodies deposited in the renal glomeruli.

Complete clinical and laboratory remissions were observed in only three cases and partial remissions were observed in other five, yet these remissions were of short duration: the mean period was six months. During the observation period rise of serum creatinine was noticed in one case in those given anti-schistosomal treatment alone and in another case among those given cyclosporine treatment. Nevertheless, improvement in kidney function as judged by the drop in serum creatinine was observed in two cases among those treated by prednisolone. Histopathologic re-evaluation showed that in most cases there were no remarkable changes and histopathologic progression of the disease was observed in four cases.

There was no toxicity related to the intake of anti-schistosomal drugs, yet hepatotoxicity and nephrotoxicity were observed in two cases in the cyclosporine treated group, and in prednisolone treated group one patient suffered from steroid related diabetes and severe upper gastrointestinal hemorrhage secondary to gastric ulcer, and another patient suffered from osteoporosis.

The prednisolone treated group showed better response than those given anti-schistosomal treatment alone and those given anti-schistosomal treatment plus cyclosporine. Nevertheless, since the remissions observed were of short duration and there was no concomitant histopathologic regression of the disease and due to the significant drug related toxicity, we conclude that none of the tried therapeutic regimens give satisfactory response if administered in the same doses for similar durations to patients who have an established disease state. Another therapeutic approach for treatment of this disease in its early and late phases should be sought.

Acknowledgment

The authors thank Mrs. Fathia Gado, Mr. Raafat Mandour, and Miss Amal Nour for their technical help, and Miss Kamilia Abu EL Naga and Hiend Sharaby for their secretarial work during preparation of the manuscript.

Reprint requests to Dr. Mohamed Sobh, Urology and Nephrology Center, University of Mansoura, Mansoura, Egypt.

References

1. Sobh M, Moustafa F, Basta M, Deelder A, Ghoneim M: Schistosomal specific nephropathy leading to end-stage renal failure. *Kidney Int* 31:1006–1011, 1987
2. Sobh M, Moustafa F, Sally S, Deelder A, Ghoneim M: Characterization of kidney lesions in early schistosomal specific nephropathy. *Nephrol Dial Transpl* 3:392–398, 1988
3. Deelder A, Klappe H, Adardweg G, Meerbeke E: *Schistosoma mansoni* demonstration of two circulating antigens in infected hamsters. *Exp Parasitol* 40:189–197, 1976
4. Deelder A, El-Dosoky I, Van Marck E, Qian Z: Immunofluorescent localization of *Schistosoma mansoni* circulating cathodic antigen in tissue of infected mice using monoclonal antibody. *Z Parasitenkd* 71:317–323, 1985
5. Andrade Z, Rocha H: Schistosomal glomerulopathy. *Kidney Int* 16:23–29, 1979
6. Andrade Z, Van Marck E: Schistosomal glomerular disease (A Review). *Mem Inst Oswaldo Cruz*, Rio de Janeiro, 79 (4):499–506, 1984
7. Hoshino-Shimizus S, De Brito T, Kanamura H, Canto A, Sliva A, Campos A, Penna D, Da Silvia L: Human schistosomiasis: *Schistosoma mansoni* antigen detection in renal glomeruli. *Trans Roy SOC Trop Med Hyg* 70:492–496, 1976
8. Houba V, Sturrock R, Butterworth A: Kidney lesions in baboons infected with *Schistoma mansoni*. *Clin Exp Immunol* 30:439–449, 1977
9. Hillyer GV: Schistosoma deoxyribonucleic acid (DNA) antibodies to DNA in schistosoma infection, and their possible role in renal pathology. *Bol Assoc Med P Rico* 66:1–22, 1973
10. Leslie T, Webster JR: Update on chemotherapy of schistosomiasis. *Bailliere Clin Trop Med Commun Dis* 2 (2): 435–447, 1987
11. Sleigh AC, Hoff R, Mott KE, Maguire JH, Da Franca Silva JT: Manson's Schistosomiasis in Brazil: 11-year evaluation of successful disease control with oxaminiquine. *Lancet* 1:635–637, 1986
12. Diaz LC de S, Pedro Rde J, Deberaldini ER: Use of praziquantil in patients with *Schistosomiasis mansoni* previously treated with oxamniquine and/or hycanthone: Resistance of *Schistosomiasis mansoni* to schistomicidal agents. *Trans Royal Soc Trop Med Hyg* 76:652–658, 1982
13. Cheever AW: A quantitative post-mortem study of schistosomiasissmansoni in man. *Am J Trop Med Hyg* 17:38–50, 1968
14. Peters PAS, Kazura JW: Update on diagnostic methods for schistosomiasis. *Bailliere's Clin Tro Med Commun Dis* 2(2):419–433, 1987