COMPARATIVE STUDIES ON DETECTING CAg IN URINE OF ACUTE SCHISTOSOMIASIS PATIENTS BY mAb-RIHA AND mAb-DotELISA

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Abstract. Urine was concentrated 20-fold for assay for CAg of *Schistosoma japonicum*. mAb-RIHA and mAb-DotELISA were positive in 78. 31% and 65.06% of cases respectively, of 83 patients with acute schistosomiasis. The false positive rates in 101 healthy controls were 14.85% and 0% , respectively. Cross-reactions (using mAb-RIHA) were seen in 16.36% and 14.28% of patients with clonorchiasis, 49 patients with ankylostomiasis, respectively. Corresponding figures for mAb-DotELISA were 0% and 0%.

INTRODUCTION

The existence of circulating antigen (CAg) in urine was first reported by Okabe and Tanaka (1958, 1961). Recently, many workers have reported the detection of CAg in serum of schistosomiasis patients and also studies on detecting CAg in urine of individuals infected with *Schistosoma mansoni, S. haematobium* and *S. intercalatum* have been reported (Ripert *et al,* 1988, 1989, 1990; De Jonge *et al,* 1989; Lieshout *et al,* 1991, 1992). However, reports on detecting CAg in urine for diagnosis of *S. japonicum* have not been made. As collection of urine is easier than that of serum and is not invasive, and the cost is little, urine-CAg assay can be used easily in the field. On the basis of animal test (Liu, 1996), we established feeze-thaw-heat concentration and used mAb-RIHA and mAb-DotELISA for detecting circulating anodic antigen (CCA) in urine of patients infected with *S. japonicum.*

MATERIALS AND METHODS

Study populations

Four groups were used in this study. Group 1, consisting of 83 patients with acute schistosomiasis were hospitalized in Wuhu. Their circumoval precipitin tests (COPT) were positive and circumoval precipitin rate were 8-48%. Among 83 cases, three consecutive duplicate 41.7 mg Kato-Katz slides were positive in 51.8%. All patients who had contact with infected water were treated with total doses 120 mg/kg praziquantel (3 times daily for 6 days); chemotherapy was effective. Group 2 consisted of 101 healthy controls from non-schistosomiasis endemic area in Meng Cheng, who had never visited a schistosomiasis endemic area and were negative by Kato-Katz thick smears. Group 3 consisted of 55 patients with clonorchiasis and 49 patients with ankylostomiasis from a non- schistosomiasis endemic area in Meng Cheng, who were negative by Kato-Katz thick smears.

Treatment of urine samples

The urine samples (30 ml/case) were stored frozen (-20°C) in sodium azide as a preservative until use. Before testing, frozen urine samples were warmed to room temperature. When the melting urine was up to 1/2 of the original sample, the solid was taken out. The melting urine (1-fold concentration) was heated in an oven for 2 hours at 76°C to remove interfering proteins and concentrated 20 fold. After centrifugation at 10,000 rpm for 5 minutes, the supernatants were assayed by mAb-RIHA. For mAb-DotELISA, urine samples did not need centrifugation.

mAb-RIHA

Monoclonal antibody 3D8A (against schistosome gut-associated antigen) was supplied by the Institute of Parasitic Diseases, Chinese Academy of Preventive Medicine, Shanghai.

6 ml 10% glutaraldehyde treated 0 human erythrocytes were washed 2 times with 0.1 M pH4.8 acetic acid-buffered saline. 200 µl monoclonal antibody 3D8A were diluted into 20 ml (100 µg/ml) with pH4.8 acetic acid-buffered saline and then 1 ml (10 µg/ml) $CrCl_3$ was added. This was heated in a 37°C water bath for 10 minutes, then 6 ml 10% 0 human erythrocytes and 30 ml of dilute tannic acid solution (1:10,000) were added immediately, mixed throughly by gently stirring and placed in a 37°C water bath for 30 minutes. After centrifuging, 1% normal rabbit serum (NRS-PBS) was added till

Table 1
Comparaision of results of detecting CAg in urine of acute schistosomiasis by two methods.

Case	No. cases examined	No.positive cases (%)	
		mAb-RIHA	mAb-DotELISA
Acute schistosomiasis cases	83	65(78.31)	54(65.06)
Healthy persons	101	15(14.85)	0(0)
Clonorchiasis sinensis cases	55	9(16.36)	0(0)
Ankylostomiasis	49	7(14.28)	0(0)

a final concentration of 1.5% hematocrit was reached. This suspension of coated erythrocytes was stored at 4°C until use. The batch number was 950228. In V-bottom microtitration plates, 25 µl samples were diluted 1:2 in PBS, after which 25 µl of 1.5% coated erythrocyes were added. After 30 minutes incubation at room temperature, results were read visually and urine samples with a titer ≥ 1:4 were identified as positive.

mAb-DotELISA

The reagent boxes were supplied by the Institute of Parasitic Diseases, Chinese Academy of Preventive Medicine, Shanghai. Urine samples were dotted on the dull side of 5x5 mm^2 x 100 nitrocellulose filter paper (0.22 µm pore size). The filters were blocked with 15 ml of 0.05% (V/V) Tween 20-PBS (0.01M pH7.4) for 30 minutes, then dried for 2 hours at 37°C. The filters were incubated with shaking at 37°C with the monoclonal antibody 3D8A diluted in 15 ml 0.05% (V/V) Tween 20-PBS (0.01 M pH7.4). After washing three times (5 minutes each) in 0.05% Tween 20-PBS, precipitable chromogenic substrates (3, 3′-Diaminobenzidine tetrahydrochloride and 4-chloro-1-naphthol) were added in PBS, activated with hydrogen peroxide and incubated for 15 minuets at room temperature. The filter was then washed three times with 0.05% PBS and read visually.

RESULTS

The original urine was given a 20-fold concentration by freezing-thawing-heating concentration. mAb-RIHA and mAb-DotELISA were positive in 78.31% and 65.06%, respectively, of 83 patients with acute schistosomiasis. The false positive rates in 101 healthy controls were 14.85 and 0%, respectively. Cross reactions (using mAb-RIHA) were seen in 16.36% and 14.28% of 55 patients with clonorchiasis, 49 patients with ankylostomiasis, respectively. Corresponding figures for mAb-DotELISA were 0% and 0% (Table 1).

DISCUSSION

The results confirmed that the gut-associated antigen of *Schistosoma japonicum* exists in urine of patients with schistosomiasis. As the quantity of CAg in urine is relatively small, urine samples have to be concentrated. To increase the quantity of CAg in urine, we established freeze-thaw-heat concentration firstly. The urine samples given a 20-fold concentration were detected by mAb-RIHA and mAb-DotELISA in parallel. The sensitivity was not high, but higer than for Kato-Katz thick smear. Further development of a simple and sensitive assay will be of great value. There are a lot of non-specific sustances in urine, so using mAb-RIHA, urine samples have to be centrifuged but the false rate was still higher. However, the detective effect of mAb-DotELISA done not be interfered by non-specific substance in urine.

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