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IMMUNODIFFUSION TESTS IN PATIENTS WITH *SCHISTOSOMA MANSONI* INFECTION

Ataualpa P. REIS [1], Naftale KATZ [2] and J. PELLEGRINO [3]

SUMMARY

Double diffusion tests by the Ouchterlony technique were performed in serum samples from 9 patients in the early stage of schistosomiasis mansoni as well as in sera from patients with chronic hepatointestinal form of the disease (67 adults and 17 children).

All patients in the chronic stage passed viable eggs in the stools and had positive complement fixation test (CFT). Sera from 10 healthy children with negative CFT for schistosomiasis were used as control.

Precipitin bands could be observed, starting from 31 days of infection, in 8 from 9 patients in the early stage of the disease (88.9%), varying in number (1 to 3) and intensity of the lines. In contrast, only 2 out of 84 patients in the chronic stage the double diffusion test was positive. All control sera were negative.

INTRODUCTION

The precipitin test has had limited application in the diagnosis of schistosomiasis. One to three precipitin bands have been reported to occur in sera from patients with active schistosomiasis mansoni when tested against homologous antigens prepared with cercariae, adult worms and eggs (KAGAN & NORMAN [5], KENT [7], BIGUET et al. [1], KRONMAN [8]). According to SILVA & FERRI [12], precipitin lines are more frequent in the hepatosplenic (78.3%) than that in the hepatointestinal (37.9%) form of schistosomiasis mansoni. In the chronic stage, DODIN et al. [3], reported 5.8% of reacting sera against adult worm (*Schistosoma mansoni*) antigen.

The present investigation was undertaken to study, by double diffusion test, the comparative reactivity of sera from patients in early as well as in chronic stage of *Schistosoma mansoni* infection.

MATERIAL AND METHODS

Serum samples — Immunodiffusion tests were performed with sera from 9 children in the early stage of *Schistosoma mansoni* infection, and with sera from patients in the chronic hepatointestinal form of the disease (67 adults and 17 children). All patients in the chronic stage passed viable eggs in the stools and had positive complement fixation test. Clinical diagnosis of the early stage of infection was based in the evidence provided by epidemiological data, clinical symptoms and laboratory tests as published elsewhere [4]. These patients have been followed up and *S. mansoni* eggs were eventually found in their feces. Sera from 10 healthy children with negative CFT for schistosomiasis were used as controls.

Antigen — It was used as antigen an extract of dried adult worms (*S. mansoni*)

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(1-3) Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais. Caixa Postal 1404, Belo Horizonte, Brazil
(2) Instituto Nacional de Endemias Rurais, Belo Horizonte, Brazil

prepared according to the following technique: After perfusion of the liver and the portal system of infected mice [11], schistosomes were washed several times in saline, quickly rinsed in distilled water, and finally lyophilized. A suspension of lyophilized adult worms in saline (1% by dry weight; 1:10,000 merthiolate as preservative) was homogenized in a tissue grinder. The suspension was left at room temperature for one hour, kept in the refrigerator overnight, and then centrifuged for 30 minutes (4°C) at 6,900 g.

The supernatant, used as antigen, contained 7.34 mg total protein/ml, determined by the method of LOWRY et al. [9]. The OUCHTERLONY [10] gel diffusion technique was performed on microscopic slides covered with 5 ml of 1% agar (Difco). Wells with 4 mm diameter and 4 mm apart were filled with antigen or human serum. Readings were made after 24 and 48 hours. Finally the preparations were washed for 5 days in saline, dried and stained with 0.4% Amido black 10-B in a 10% solution of acetic acid.

RESULTS

The results of immunodiffusion tests performed with sera from patients in the early stage of schistosomiasis are shown in Table I. Precipitin bands could be observed, starting from 31 days of infection, in 8 from 9 patients (88.9%) varying in number and intensity. In patients with chronic hepatointestinal form, only 2 reactors out of 84 were detected, both showing only one precipitin band. In the 10 control sera, no precipitin bands were observed.

TABLE I

Double diffusion tests in patients with early schistosomiasis mansoni

Patients	Probable duration of the infection (days) at the time tests were made	Precipitin bands (*)		
		A	B	C
1	30	0	0	0
	55	0	0	0
2	31	+	0	0
	75	++	+	0
3	37	+	+	0
4	48	+	0	0
	75	+++	0	0
5	58	+	0	0
	75	+++	0	0
6	63	+	++	0
	158	0	++	0
7	65	+	+	0
	94	+	+	0
8	65	++	+	+
	94	++	0	+
9	65	0	0	0
	94	++	0	0

(*) 0 = no precipitin band. The intensity of precipitin bands were graded from + to +++

DISCUSSION

KAGAN & NORMAN [5] reported that sera from patients with proved *Schistosoma mansoni* infection, when tested against antigen prepared from adult worms, cercariae, and eggs, by the agar-gel diffusion method, 1 to 3 precipitin bands occurred in 13 out of 29 sera. KENT [7] testing 35 schistosome sera (neither stages nor clinical forms of the disease were mentioned in the paper) against adult worm and cercaria antigens by the Ouchterlony technique found 2 to 3 bands in most cases. Using adult worm antigen DODIN et al. [3] found 5.8% reactors in 86 sera from chronic schistosomiasis mansoni patients, with 1 to 2 bands. However, after treatment, 4 to 8 precipitation lines were observed in 91% of cases. Double diffusion tests using adult worm antigen were performed by SILVA & FERRI [12] in 66 patients (29 with the hepatointestinal form and 37 with hepatosplenic form). Precipitin lines were more frequent in the hepatosplenic (78.3%) than in the hepatointestinal form (37.9%). The number of precipitin bands varied from 1 to 4 in patients with hepatosplenic form, whereas only one antigen-antibody system was generally observed in the hepatointestinal cases of schistosomiasis mansoni. These observations were confirmed by CAPRON et al. [2] and the differences in the pattern of reactivity of sera according to the clinical stages of the disease were also emphasized.

The results obtained in our studies clearly show that precipitin bands are far much more frequent at the early stages of *S. mansoni* infection. On the other hand, immunodiffusion may be used as a complementary test for the diagnosis of the disease in conjunction with other immunosorological tests (KAGAN & PELLEGRINO [6]).

RESUMO

Teste de imunodifusão em pacientes com esquistossomose mansônica

Foram realizados testes de dupla difusão em ágar (técnica de Ouchterlony) com soros de 9 crianças na fase aguda de esquistossomose mansônica, assim como também com soros de 84 pacientes na fase crônica (67 adultos e 17 crianças; forma hepatointestinal).

Todos os pacientes na fase crônica possuiam ovos viáveis de *S. mansoni* nas fezes e reação de fixação do complemento positiva. Soros de 10 crianças sadias e com reação de fixação do complemento negativa para esquistossomose foram usados como contrôle.

Linhas de precipitação foram observadas, a partir de 31 dias de infecção, em 8 dos 9 pacientes na fase aguda (88,9%), variando em número (1 a 3) e intensidade. Ao contrário, em apenas 2 soros dos 84 pacientes na fase crônica o teste de Ouchterlony foi positivo, com uma só linha de precipitação. Os soros tomados como contrôle foram negativos.

Os dados apresentados mostram que o teste de imunodifusão em ágar pode ser útil para o diagnóstico da esquistossomose mansoni em sua fase inicial.

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