Rev. Inst. Med. trop. São Paulo
7(1):1-6, janeiro-fevereiro, 1965

UDC 616.995.122

IMMUNODIFFUSION STUDIES IN HUMAN SCHISTOSOMIASIS MANSONI

I. Hepatointestinal and hepatosplenic forms

Luiz Caetano da SILVA [1] and Rubens Guimarães FERRI [2]

SUMMARY

Sera from 66 patients with Schistosomiasis mansoni (29 with the hepatointestinal form and 37 with the hepatosplenic form) were studied by the OUCHTERLONY technique.

All of the patients with the hepatointestinal form and 23 patients with the hepatosplenic form had viable eggs in the stools.

Double-diffusion tests showed that precipitin lines are more frequent in the hepatosplenic (78.3%) than in the hepatointestinal forms (37.9%). The number of precipitin lines varied from one to four in the hepatosplenic form whereas only one antigen-antibody system was generally observed in the hepatointestinal form.

Differences between both forms and the importance of the presence of viable eggs in the stools are discussed.

INTRODUCTION

Most immunodiffusion studies in Schistosomiasis mansoni are related to sera from immunized [1, 2, 11] and experimentally infected animals [3, 10, 11].

Human cases have been studied by BIGUET et al. [1], KAGAN et al. [2] and KENT [3] using the OUCHTERLONY technique.

Precipitin lines were demonstrated in the majority of the cases by diffusing sera against *Schistosoma* antigens. Differences between hepatointestinal and hepatosplenic forms and the correlation of the test with the presence of viable eggs in stools, have not been suficiently emphazised.

The present investigation was undertaken to study sera from patients with either form of schistosomiasis by the OUCHTERLONY technique. Highly positive sera were further submited to immunoelectrophoresis in order to localize precipitin lines. The results of these studies are being published in this issue [9].

MATERIAL AND METHODS

Sera from 66 patients with Schistosomiasis mansoni (29 with the hepatointestinal form and 37 with the hepatosplenic form) were studied by the OUCHTERLONY technique [5].

Diagnosis was based on the presence of viable eggs in the stools or in fragments of

This research was supported in part by a grant from "Fundação de Amparo à Pesquisa do Estado de São Paulo".

(1) Division of Liver Diseases, Instituto de Medicina Tropical de São Paulo (Prof. A. D. F. Amaral). Departamento de Clínica Médica (Prof. L. V. Décourt), Hospital das Clínicas, São Paulo, Brazil. Instituto de Gastrenterologia de São Paulo (Prof. J. F. Pontes).

(2) Division of Immunochemistry, Departamento de Microbiologia e Imunologia (Prof. C. S. Lacaz), Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil.

rectal biopsy. Some negative cases of the hepatosplenic form were also included. Its diagnosis was based on either of the following criteria:

a) presence of inviable eggs; b) previous positive stool examinations; c) diagnosis by liver biopsy. Thus, four groups of patients were studied:

GROUP I: Hepatointestinal form with viable eggs (29 patients).

GROUP II: Hepatosplenic form with viable eggs (23 patients).

GROUP III: Hepatosplenic form without viable eggs (14 patients).

GROUP IV: Control group constituted by 6 normal adults, 3 patients with liver cirrhosis, 2 with malaria, one with South American blastomycosis and one with eggs of *Hymenolepis nana* in the stools. Serum from one mouse with *H. nana* was also included.

In every case the antigen used was extracted from adults worms, according to the following technique.

After perfusion of the liver and portal system of infected guinea-pigs [2] and mice[3], worms were carefully washed in saline (NaCl 0.85%) and only once in distilled water and finally dried under vaccum at minus 20°C or lyophilized.

A 3% suspension of worms in saline (0.85% of NaCl with 1:10,000 of merthiolate) was homogenized for 5 minutes in "Virtis Homogenizer" under refrigeration with a salt-ice mixture, at approximately 40,000 r.p.m. The homogenate was kept at 4°C overnight and then centrifuged.

The protein content of the supernatant was determined by the method of LOWRY et al. [4] and varied from 700 to 850 mg per 100 ml.

The OUCHTERLONY [5] gel diffusion method on microscopic slides was used, the diffusion being carried out for 48 hours. Three ml of a 1% of agar solution (in 0.85% of NaCl with 1:5,000 of merthiolate) was used. Wells with 4 mm in diameter and 4 mm apart were filled with antigen or human serum.

Staining was performed with 0.4% Amido Black 10B in a 10% solution of acetic acid, after washing and drying of the gel plates.

RESULTS

Hepatointestinal forms (Group I): precipitation lines were observed in 11 out of 29 cases (37.9%) (Table I).

Hepatosplenic forms (Groups II and III): precipitation lines were observed in 29 out of 37 cases (78.3%).

Twenty cases from Group II (86.9%) and 9 from Group III (64.3%) showed antibodies in the serum by double-diffusion test (Table II).

Precipitation patterns (Fig. 1 and 2). Besides the higher positivity of hepatosplenic form as compared to hepatointestinal form, the number of lines varied from one to four in Groups II and III whereas only one antigen-antibody system was generally observed in Group I (Tables I and II).

Control cases (Group IV): no precipitation lines were observed, showing that nonspecific reactions were absent.

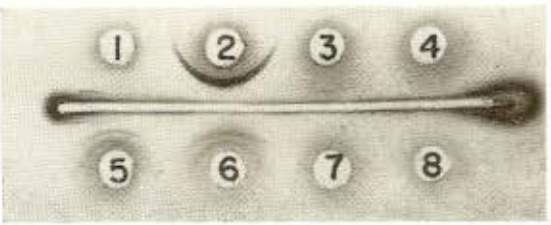

Fig. 1 — Double diffusion test in sera from patients with the hepatosplenic form (No. 1 to 5) and with the hepatointestinal form (No. 6 to 8).

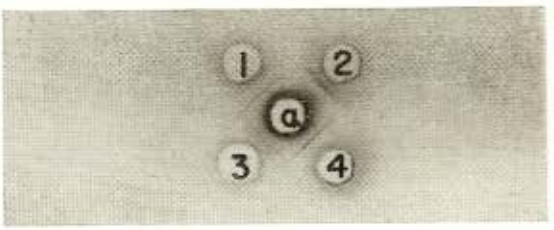

Fig. 2 — Double diffusion test in sera from patients with the hepatosplenic form (No. 1 and 4) with the hepatointestinal form (No. 2) and from immunized rabbit (No. 3).

TABLE I

Gel-double-diffusion tests in hepatointestinal forms of Schistosomiasis (Group I) *

Patient no.	Age-Sex	Viable eggs	Precipitation lines	
			Intensity **	Number of lines
1 — J.J.S.	45 years — ♂	Present	+	1
2 — P.M.	38 years — ♂	"	++	1
3 — D.B.S.	29 years — ♀	"	+	1
4 — R.P.S.	19 years — ♂	"	+	1
5 — A.A.B.	36 years — ♂	"	+	1
6 — J.P.P.	11 years — ♂	"	++	1
7 — G.F.	32 years — ♂	"	++	2
8 — J.M.C.	6 years — ♂	"	+	1
9 — M.C.C.	24 years — ♀	"	+	1
10 — A.S.L.	17 years — ♂	"	+	1
11 — J.O.M.	34 years — ♂	"	++	1

* Negative precipitin tests in 18 sera from patients with viable eggs were not included

** Graded from + to +++

TABLE II

Gel-double-diffusion tests in hepatosplenic forms of Schistosomiasis with and without viable eggs (Groups II and III, respectively) *

Patient no.	Age-Sex	Viable eggs	Precipitation lines	
			Intensity **	Number of lines
1 — S.C.N.	38 years — ♀	Present	+	1
2 — A.B.B.	40 years — ♂	"	+	1
3 — M.M.O.	37 years — ♂	"	+	1
4 — J.A.C.S.	24 years — ♀	"	+	1
5 — J.P.	24 years — ♂	"	++	3
6 — M.C.	35 years — ♀	"	++	2
7 — R.F.	28 years — ♀	"	++	2
8 — J.F.S.	16 years — ♂	"	+	2
9 — A.F.C.	11 years — ♂	"	+	1
10 — J.B.N.	10 years — ♂	"	+	2
11 — E.F.M.	19 years — ♂	"	++	4
12 — M.C.J.	40 years — ♀	"	++	2
13 — R.R.F.	43 years — ♂	"	+	2
14 — S.P.	18 years — ♂	"	++	2
15 — M.P.S.	33 years — ♀	"	+	1
16 — E.S.B.	35 years — ♂	"	+	1
17 — B.F.	26 years — ♂	"	+	1
18 — L.S.	40 years — ♂	"	+	2
19 — C.M.S.	28 years — ♂	"	+	2
20 — A.R.V.	10 years — ♀	"	+	2
21 — A.S.	55 years — ♀	Absent	++	2
22 — J.A.M.	32 years — ♂	"	++	2
23 — D.A.D.	35 years — ♀	"	+	1
24 — R.V.	20 years — ♂	"	+	2
25 — I.G.S.	34 years — ♀	"	++	2
26 — D.S.	40 years — ♂	"	++	1
27 — G.M.A.	26 years — ♀	"	++	2
28 — A.F.S.	26 years — ♂	"	+++	2
29 — J.S.	16 years — ♂	"	+	2

* Negative precipitin tests (3 cases from Group II and 5 cases from Group III) were not included

** Graded from + to +++

DISCUSSION

Considering the high positivity of some tests as complement fixation, flocculation and immunofluorescence tests [6], our results show that immunodiffusion is not so satisfactory for the diagnosis of *Schistosomiasis mansoni*, particularly of the hepatointestinal forms.

It must be pointed out, however, that our results are by no means definitive and that immunodiffusion tests were not performed with diagnostic purposes, but for the selection of strongly positive sera in order to study the characteristics of the antibodies [9].

Using water extracts of adult and cercarial forms of *Schistosoma mansoni*, KENT [5] detected two or three antibodies in most human sera but he did not mention the clinical forms.

KAGAN et al. [4] tested sera from 29 patients, some of them were suspected and others proved cases of schistosomiasis. Only 17 had eggs in their stools. They found 13 sera reacting with antigen of adult worms, 14 sera with cercarial antigen and 10 with egg antigen. No more than three bands were present in any reaction and usually only one. No mention was made to the clinical forms of schistosomiasis.

Using adult worm antigen BIGUET et al. [1] found precipitation lines in 28 out of 32 sera. The authors, however, did not mention the presence or absence of eggs in the stools and the clinical forms.

Our results show that the positivity in the whole group of patients was rather low (60.6%). However, it is worth pointing out the differences of positivity among the three groups, particularly between Groups I and II (respectively hepatointestinal and hepatosplenic forms, both with viable eggs).

The differences of methods and the lack of information about the clinical features of the patients studied by the above mentioned authors do not allow any comparison among our and their results.

We wonder whether a higher percentage of positivity should be obtained in our material with the use of other antigens. As a matter of fact, KENT [3] has emphazised the advantages of employing deffated water extracts of adult and cercarial forms of *Schistosoma mansoni.*

As far as cross-reactions are concerned, we did not observe precipitin reactions with sera from 2 patients with malaria, one with South-American blastomycosis and one with *Hymenolepis nana* eggs. The possibility exists that such cross-reactions might be observed if more patients with these and other diseases and different types of antigens were used. KENT [3] showed that reactions between *Schistosoma* antigens and anti-trichinella sera from natural infections as well as sera obtained from actively immunized animals may be observed and that the antigen responsible is heat-labile.

Finally, it is worth mentioning the differences in the number of precipitin bands, between the hepatointestinal and hepatosplenic forms. Our results show that antibody systems are more complex and more easily demonstrated by immunodiffusion in hepatosplenic form, particularly in the serum of patients with eggs in their stools.

RESUMO

Estudos sôbre imunodifusão na esquistossomose mansônica humana. I — Formas hepatintestinal e hepatesplênica.

Foram estudados pela técnica de OUCHTERLONY soros de 66 pacientes com esquistossomose mansônica, dos quais 37 com a forma hepatesplênica e 29 com a forma hepatintestinal.

Todos os pacientes com a forma hepatintestinal e 23 com a forma hepatesplênica apresentavam ovos viáveis nas fezes.

Os testes de dupla difusão em ágar mostraram que as linhas de precipitação são mais freqüentes na forma hepatesplênica (78,3%) que na hepatintestinal (37,9%). O número de linhas de precipitação variou de uma a quatro na forma hepatesplênica, enquanto apenas uma linha foi observada na maioria dos casos da forma hepatintestinal.

São discutidas as diferenças entre ambas as formas e a importância da presença de ovos viáveis nas fezes.

ACKNOWLEDGMENTS

Part of the technical assistance was possible thanks to the "Instituto Brasileiro de

Estudos e Pesquisas de Gastrenterologia" (IBEPEGE). The writers are grateful to Dr. W. LOBATO PARAENSE and to Dr. JOSÉ PELLEGRINO (Instituto Nacional de Endemias Rurais) for supplying the infected snails and part of the lyophilized worms. Special thanks are due to HELENA SAEKO ITANO, SUMIE HOSHINO and TIYO TUTYIA MATSUO, for their technical assistance in these studies.

REFERENCES

1. BIGUET, J.; CAPRON, A. & TRAN VAN KY, P. — Les antigènes de Schistosoma mansoni. I — Étude électrophorétique et immunoélectrophorétique. Caractérisation des antigènes spécifiques. *Ann. Inst. Pasteur* (Paris) **103**:763-777, 1962.

2. KAGAN, I. G. & NORMAN, L. — Analysis of helmint antigens (Echinococcus granulosus and Schistosoma mansoni) by agar gel methods. *Ann. New York Acad. Sc.* **113**:130-153, 1963.

3. KENT, N. H. — Comparative immunochemistry of larval and adult forms of Schistosoma mansoni. *Ann. New York Acad. Sc.* **113**:100-113, 1963.

4. LOWRY, O. H.; ROSEBROUGH, N. J.; FARR, A. L. & RANDALL, R. J. — Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**:265-275, 1951.

5. OUCHTERLONY, O. — In vitro method for testing the toxin producing capacity of diphtheria bacteria. *Acta path. et microbiol. scandinav.* **25**:186-191, 1948.

6. PELLEGRINO, J. — Diagnóstico sorológico da esquistossomose mansônica. I. Estudo comparativo entre as reações de floculação, de imunofluorescência e de fixação do complemento. *Rev. Inst. Med. trop. São Paulo* **5**:147-153, 1963.

7. PELLEGRINO, J. & SIQUEIRA, A. F. — Técnica de perfusão para colheita de *Schistosoma mansoni* em cobaias experimentalmente infestadas. *Rev. Brasil. Malariol.* **8**: 589-579, 1956.

8. SILVA, L. C. da — *Unpublished data.*

9. SILVA, L. C. da & FERRI, R. G. — Immunodiffusion studies in human Schistosomiasis mansoni. II. Localization of antibodies by immunoelectrophoresis. *Rev. Inst. Med. trop. São Paulo* **7**:7-10, 1965.

10. SMITHERS, S. R. — Gel-diffusion studies on Schistosoma mansoni. *Tr. Roy. Soc. Trop. Med. & Hyg.* **54**:8, 1960.

11. SMITHERS, S. R. — Stimulation of acquired resistance to *Schistosoma mansoni* in monkeys: role of eggs and worms. *Exper. Parasit.* **12**:263-273, 1962.

Recebido para publicação em 3/12/1964.