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Detection of Antibodies to *Schistosoma japonicum* Ova in Schistosomiasis Patients by Gelatin Agglutination Test

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Abstract

In this study, a new serological test, gelatin agglutination test (GAT) was used for the detection of antibodies to *Schistosoma japonicum* ova being compared with latex agglutination test (LAT) and enzyme-linked immunosorbent assay (ELISA). In 156 sera from patients with schistosomiasis japonica, 150 (96.1%) were positive; in 13 sera from patients with acute schistosomiasis japonica, all were positive; while in 30 sera from healthy individuals, only one was weakly positive. One of 15 sera from patients with clonorchiasis sinensis was positive; all of 10 sera from paragonimiasias westermani were negative. In 4th and 8th month after chemotherapy, 20 patients with schistosomiasis japonica showed decreased serum titers at different degrees ($p<0.01$). The results of GAT were comparable to those of LAT and ELISA. GAT was simple, rapid and inexpensive. The results could be recognized easily by the naked eye. It appeared to be one of the useful screening methods for the diagnosis and seroepidemiological survey of schistosomiasis especially in fields.

Key words: schistosomiasis japonica, gelatin agglutination test (GAT), latex agglutination test (LAT), ELISA

Introduction

Many modern immunological techniques, such as radioimmunoassay (RIA), FAST-ELISA, Western blotting have been used for the diagnosis and seroepidemiological study of schistosomiasis (Mott *et al.*, 1987; Pelley *et al.*, 1977; Hancock and Tsang, 1986; Ruppel *et al.*, 1985). However, we still lack rapid, simple and sensitive methods suitable for field application.

Recently, a new kind of agglutination test with the application of newly developed gelatin particles has been used to detect antibodies specific for human immunodeficiency virus (Yoshida *et al.*, 1986; Constantine *et al.*, 1989), human sperm (Dondero *et al.*, 1991) and for the diagnosis of human strongyloidiasis (Sato and Ryumon, 1990). In this study, we tried to apply gelatin agglutination test (GAT) to detect antibodies specific for *S. japonicum*. The results were compared with those of latex agglutination test (LAT) and enzyme-linked immunoabsorbent assay (ELISA).

Materials and Methods

1. Sera

A total of 325 sera were collected from inhabitants in one of the endemic areas of schistosomiasis japonica in Meishan Town of Sichuan Province, China. Among them, 156 sera were from the positive for schistosome ova, while 169 sera were from the negative for schistosome ova by the stool examination (Kato-Katz's method). In Jiangpu County of Jiangsu Province, China, 20 sera were collected from the patients with schistosomiasis japonica proved also by the stool examination before chemotherapy, in 4th and 8th month after chemotherapy (praziquantel 30 mg/day/kg for 2 days). In Nanjing, Jiangsu Province, 13 sera were collected from the

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patients with severe acute schistosomiasis japonica. All of the 13 patients had fever and other clinical features of acute schistosomiasis with positive results of the stool examination. As negative control, 15 sera from patients with clonorchiasis sinensis and 10 sera from paragonimiasis westermani were collected from non-endemic areas of schistosomiasis in Jiangsu. Also 30 sera from healthy individuals in Yamanashi Prefecture, Japan were used as negative control.

2. Antigen preparation

Soluble egg antigen (SEA) was prepared from eggs of *S. japonicum*. Eggs were isolated from livers of experimentally infected rabbits. The eggs were ground with an earthen mortar and then sonicated for one min. The suspension was frozen with dry ice and thawed for three times, stirred at 4°C overnight, and then, it was centrifuged at 10,000g for 30 min. The supernatant was aliquoted and stored at –80°C.

3. Antigen coating

The optimum concentration of antigen solution was determined by Box titration test, using pooled serum of infected chinese patients.

The gelatin particles were kindly supplied from Prof. Y. Sato, University of the Ryukyus School of Medicine, Okinawa, Japan and GAT was done according to the method by Sato and Ryumon (1990) with slight modifications. Gelatin suspension was washed three times (3,000rpm, 5 min.) with PBS (pH7.2, 0.05 M) and then treated with 10^{-4} tannic acid dissolved in PBS for 30 min at room temperature. After washing, it was adjusted to 3% concentration with normal saline. The suspension was mixed with equal volume of SEA (300 μg/ml) at 37°C for 1.5 hr. Then it was washed three times and adjusted to 1% concentration with PBS containing 1% bovine serum albumin.

The latex particles (SDL-48GE, the Takeda Pharmaceutical Industrial Co., Ltd) were washed two times (4,000rpm, 20 min) with glycine buffer (GB, pH8.2, 0.05M) and adjusted to 2% concentration. The suspension was mixed with equal volume of SEA solution (500 μg/ml), kept at 37°C for 1 hour with stirring every 5 min. Then it was washed two times and adjusted to 0.1% concentration with GB containing 0.5% bovine serum albumin.

4. Agglutination test

The test sera were also diluted in the wells of microplates in a serial 2-fold dilutions. Then 25 μl diluted serum and 25 μl suspension of antigen coated particles were placed into each well and mixed. The particles were allowed to settle for 3 hr, then the setting patterns at the bottoms were read by the naked eye.

5. ELISA

ELISA was followed with the indirect method by Nakao *et al.* (1981). The substrate used was O-phenylenediamine (OPD). For each sample serum, four 2-fold dilutions (80–640) were assayed. The correlation coefficients and regression equations between OD values and the dilution times of sera were calculated by a computer. When OD equals 0.2, the dilution times (termed as titers) were obtained according to the equation also by the computer.

6. Statistics

Associations of GAT with LAT and ELISA were analysed by linear correlation and regression. The statistical significances of the differences between groups before and after chemotherapy were analysed by the paired t-test after the titers of GAT, LAT and ELISA were transformed into logarithms ($\log_2$ or log).

Results

1. Effects of preserving conditions of antigen-coated gelatin and latex particles on the results:

The antigen-coated gelatin and latex particles were kept at 4°C, frozen or lyophilized and kept at –80°C for 6 months. The antibody titers of 10 sera from patients with schistosomiasis and 5 sera from healthy individuals were detected with these particles compared with newly prepared ones (Fig. 1). For GAT, the results of both frozen and lyophilized particles were similar to those of newly prepared ones; while, preservation of particles at 4°C influenced the results leading to slightly decreased titers. For LAT, the above three kinds of preserved particles presented similar results to newly prepared ones.

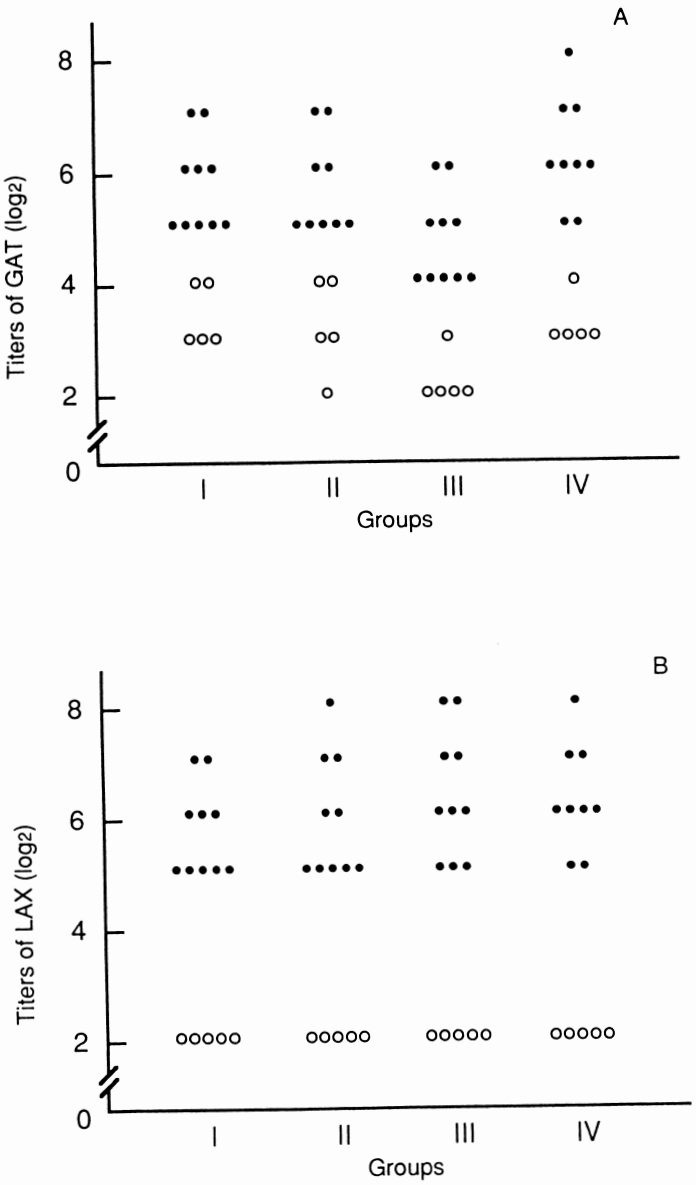

Fig. 1 Effects of preserving conditions of antigen-coated gelatin (A) and latex particles (B) on the results. Solid circles: sera from the patients with schistosomiasis. Open circles: sera from the healthy individuals. The antigen-coated particles were lyophilized (group I) or frozen (group II) and then kept at –80°C, or the particles were only kept at 4°C (group III) for the period of six months with comparison to newly prepared ones (group IV).

2. Detection of antibodies to *S. japonicum* in different human groups:

GAT, LAT and ELISA were used to detect antibodies to *S. japonicum* in four groups including inhabitants in one of the endemic areas in Sichuan (group A, B), patients with acute schistosomiasis (group C) and healthy individuals (group D) (Tables 1–3). In GAT and LAT, the serum titers were equal

Table 1 Distribution of GAT titers in different groups

Groups	No. tested	GAT titers (log_2)									
		≤3	4	5	6	7	8	9	10	11	≥12
A	156	1	5	4	51	42	12	2			
B	169	27	31	47	36	18	5	2			3
C	13				1				2	3	7
D	30	27	2	1							

Group A: Inhabitants with the positive result of stool examination in Sichuan Province, China
B: Inhabitants with the negative result of stool examination in the same area as group A
C: Patients with acute schistosomiasis japonica in Nanjing, Jiangsu Province, China
D: Healthy individuals in Yamanashi Prefecture, Japan.

Table 2 Distribution of LAT titers in different groups

Groups	No. tested	LAT titers (log_2)									
		≤3	4	5	6	7	8	9	10	11	≥12
A	156	3	6	40	47	38	16	6			
B	169	32	24	39	34	24	11		2		3
C	13					1			1	2	9
D	30	28	2								

Table 3 Distribution of ELISA titers in different groups

Groups	No. tested	ELISA titers (log)						
		<2.0	~2.5	~3.0	~3.5	~4.0	~4.5	~5.0
A	156	2 (1.3)	35 (22.4)	63 (40.0)	37 (23.7)	13 (8.3)	5 (3.2)	1 (0.6)
B	169	36 (21.3)	76 (45.0)	41 (24.3)	11 (23.7)	5 (3.0)		
C	13				1 (7.7)		2 (12.4)	10 (77.0)
D	30	29 (96.7)	1 (3.3)					

( ): %.

to or over (≥) 1:32 in most people in group A and in over 65% of group B, and in all of the patients in group C. While, almost all the sera gave negative agglutination at the dilution of 1:32 in control group.

From these results, the criteria of the GAT and LAT were estimated as follows: the serum titer ≥1:32 (log_2 titer ≥5), positive; 1:16 (log_2 titer =4), equivocal; ≤1:8 (log_2 titer ≤3), negative. In ELISA, sera

with titers equal to or over 1:100 (log titer ≥2) were estimated positive. The positive numbers and rates in each group were shown in Table 4.

Associations of GAT with LAT and ELISA were analysed in the sera from 156 schistosome-ova-positive inhabitants (group A) and 13 patients with acute schistosomiasis (group C) totaled 169. The correlation coefficient of GAT with LAT was 0.870; and that with ELISA was 0.742, respectively (Figs. 2, 3).

3. Changes of serum titers after chemotherapy:

Sera from 20 patients with schistosomiasis japonica were detected by GAT, LAT and ELISA before and after chemotherapy. Compared with the levels before chemotherapy, the serum titers were decreased in 4th month and further decreased in 8th month after chemotherapy in most cases (Fig. 4). The changes were statistically significant ($p<0.01$).

Table 4 The positive numbers and rates in each group with the methods to detect of antibodies to *S. japonicum*

Groups	No. tested	GAT		LAT		ELISA	
		Positive	%	Positive	%	Positive	%
A	156	150	96.1	147	94.2	154	98.7
B	169	111	65.7	113	66.9	133	78.7
C	13	13	100.0	13	100.0	13	100.0
D	30	1	3.3	0	0	1	3.3

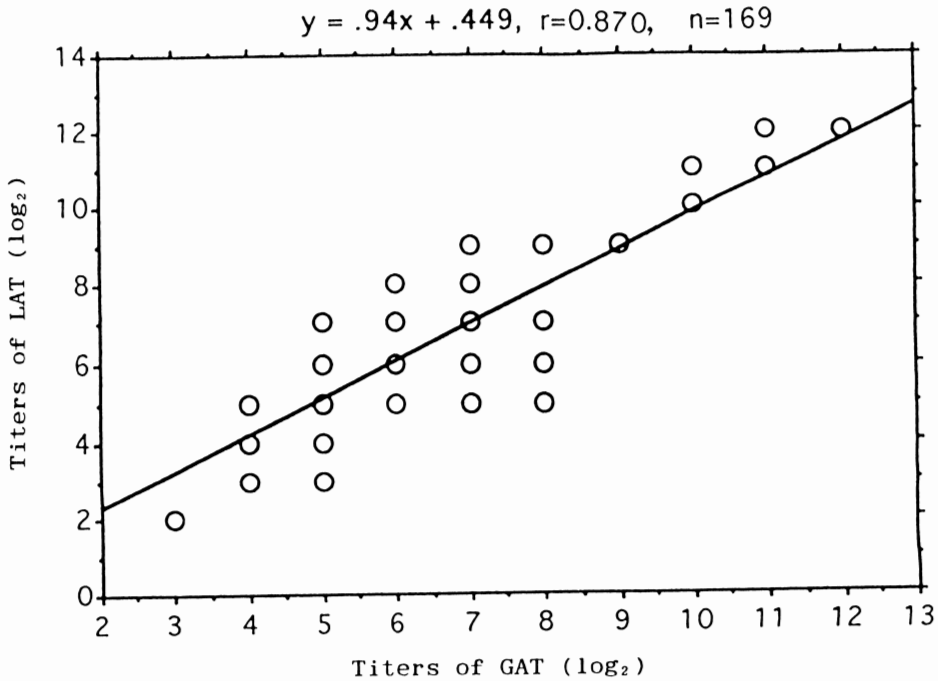

Fig. 2 Correlation between GAT and LAT, in the sera from group A (156 inhabitants with the positive result of stool examination) and C (13 patients with schistosomiasis japonica) totaled 169. The correlation coefficient of GAT with LAT was 0.870.

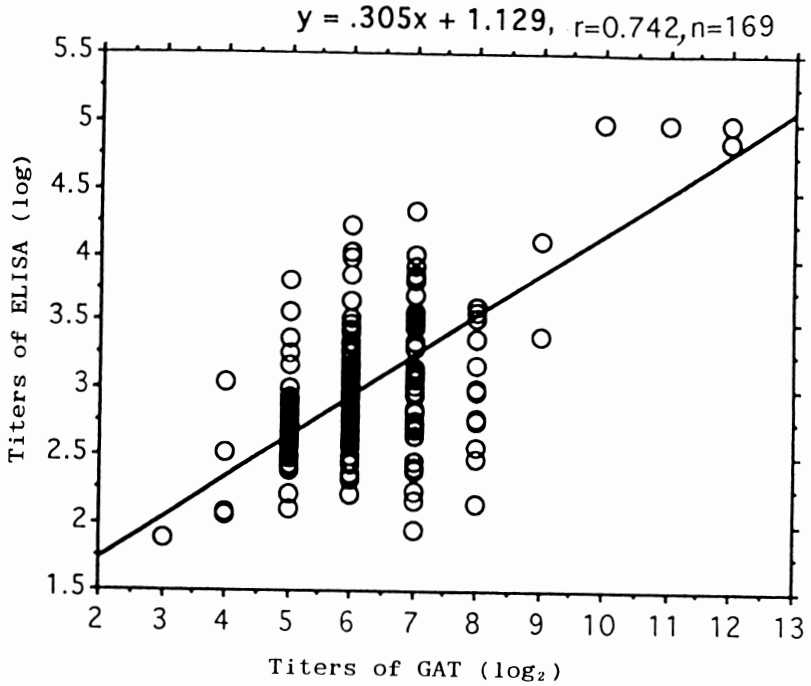

Fig. 3 Correlation between GAT and ELISA. The correlation coefficient of GAT with ELISA was 0.742. The sera tested were the same as denoted in Fig. 2.

4. Cross-reactions:

Sera from 15 patients with clonorchiasis and 10 patients with paragonimiasis were detected by GAT, LAT and ELISA. Only one of the sera from clonorchiasis was positive by GAT with titer 1:32, while all were negative by LAT and ELISA. For the sera from paragonimiasis, no serum was positive by GAT, LAT or ELISA.

Discussion

Traditionally, schistosomiasis has been diagnosed by direct parasitological techniques, such as stool examination, miracidial hatching test. These techniques are generally laborious, time-consuming and insensitive especially in areas with low intensity of the infection (Mott and Dixon, 1982; Bergquist, 1992). Furthermore, the high day-to-day fluctuations in egg passage necessitates multiple egg counts to obtain reliable results (Polderman, 1985). As the present findings showed, 65.7% of the sera from group B were positive by GAT. There is an urgent need to develop simple, fast and reliable diagnostic techniques for field application.

The widely used immunodiagnostic techniques for schistosomiasis include ELISA, circumoval precipitin test (COPT), indirect hemagglutination test (IHAT) etc. However, ELISA relies on specific equipments, and some expensive reagents. Its procedure is complicated and time-consuming. It seems unsuitable for field application. COPT and IHAT are simple and do not need specific apparatuses. But the sensitivity of COPT is low, besides it takes 48–72 hr to obtain the results. The stability of IHAT is unfavourable.

Sato and Ryumon (1990) used gelatin agglutination test for sero-diagnosis of human strongyloidiasis. Their results obtained were quite sensitive and specific for diagnosis showing a close correlation with those of the IHAT and ELISA. They believed that the test should be more suitable for mass screening for *Strongyloides* infection than the IHAT and the ELISA. In this study, we tried to use newly developed gelatin particles as an antigen carrier for indi-

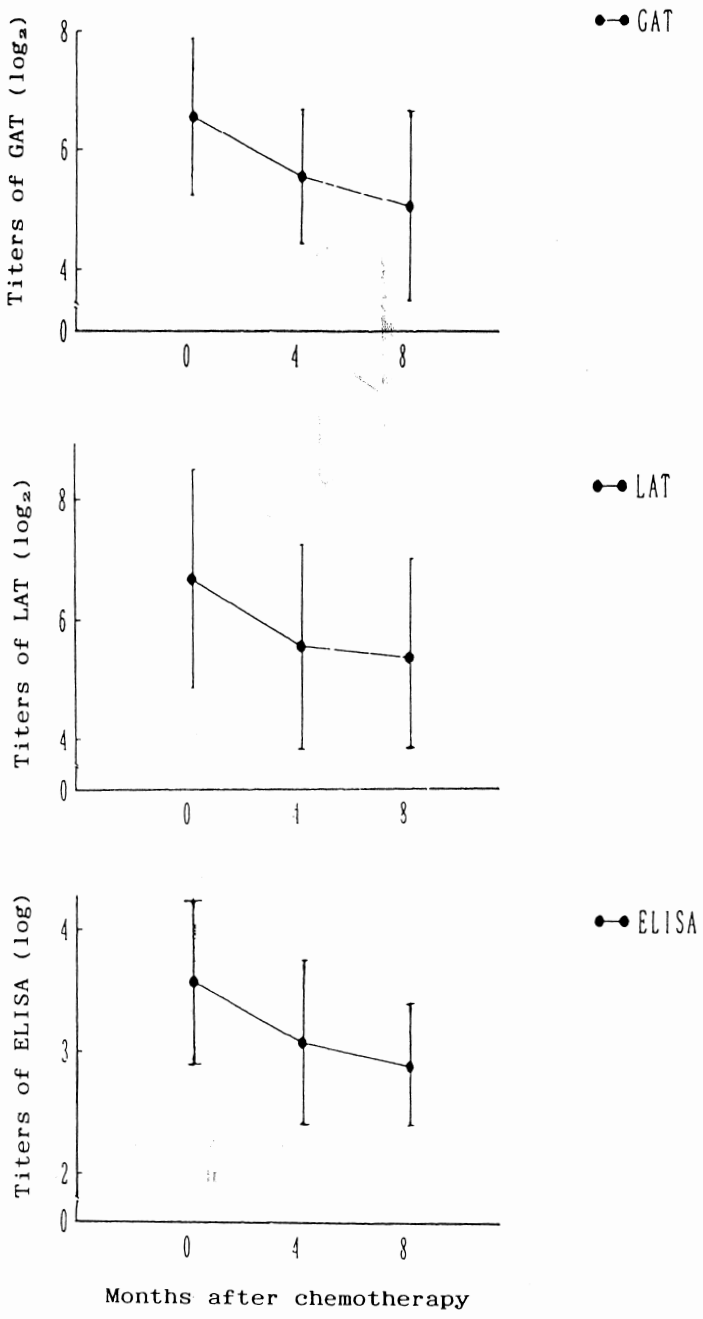

Fig. 4 Changes of serum titers after chemotherapy. The changes were statistically significant ($p<0.01$).

rect agglutination test for schistosomiasis japonica. The results of GAT was comparable to those of LAT and ELISA, although the preservation of antigen-coated gelatin particles at 4°C for 6 months led to slightly decreased titers. The antigen, 2.5 μg of SEA in GAT and 0.625 μg in LAT and 0.875 μg in ELISA was used for each well. In the present study, more antigen was required for GAT than for LAT or ELISA; which is the disadvantage of GAT. Further improvement of antigen coating seems to be necessary. However, the procedure of GAT was very simple, involved only one step. The result could easily be read 3 hr later by the naked eye. The antigen-coated gelatin particles could be lyophilized and stored for long time. The cross-reactions with other trematodes were infrequent. GAT appeared to be one of the useful screening methods for the diagnosis and epidemiological survey of schistosomiasis especially in fields.

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