THE GELATIN PARTICLE INDIRECT AGGLUTINATION TEST, A MEANS OF SIMPLE AND SENSITIVE SERODIAGNOSIS OF CHAGAS' DISEASE

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Abstract: An indirect agglutination test using a *Trypanosoma cruzi* antigen-coated gelatin particles was employed to diagnose trypanosomiasis in Paraguay. Results with this test were quite comparable to those obtained with enzyme-linked immunosorbent assay (ELISA). Furthermore, nonspecific reaction to the gelatin particles alone was not found in either acute or chronic infection. This method is more convenient than the ELISA, since the antigen-conjugated particles is stable for at least 1 year at 4°C and since the test itself is short and simple to perform and does not require specialized equipment.

INTRODUCTION

Chagas' disease is a chronic parasitosis caused by *T. cruzi*. It is a serious health problem in Latin America where estimated 16-18 million people are infected and 100 million are at a risk of infection (WHO report, 1991). Acute Chagas' disease is generally characterized by fever, chagoma, blood parasitemia, lymphadenopathy and a low level of antibodies in serum. A few patients die during the acute phase. Some resolve to the chronic phase in which fatal cardiomyopathy, megacolon, megaesophagus and neuropathies may occur despite the relative absence of routinely demonstrable parasites in their blood or tissues (Garry and David, 1992), although high levels of antibodies have been detected (Krettl, and Brener, 1976).

T. cruzi infection is usually diagnosed by serological methods, except in the acute stage, where parasite isolation is feasible. The polymerase chain reaction (PCR) has been applied to the diagnosis of Chagas' disease (Russomando *et al.*, 1992) as have serological tests such as complement fixation (File and

Kent, 1960), immunofluorescence (Camargo, 1966), hemagglutination (Camargo, 1973) and ELISA (File and Kent, 1960). ELISA and PCR have come into more common use because of their greater sensitivity. However, for ELISA and PCR, specialized materials and reagents such as enzyme-coupled antibodies, micro-ELISA reader, DNA synthesizer, analyzer and primer are required. Recently, however, an indirect agglutination method using a gelatin particles was used in a screening survey of strongyloidiasis by Sato *et al.*, (1990). This test is sensitive, technically simple and can be performed rapidly without specialized equipment or facilities. This makes it useful for screening and serological diagnosis in endemic areas, especially those in which the electric power supply is unstable. In light of this, we attempted to evaluate whether this method could be used instead of ELISA for the serodiagnosis of Chagas' disease in endemic areas.

MATERIALS AND METHODS

Subjects

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Serum samples were obtained from 40 Paraguayan subjects. Informed consent was obtained from all patients before their involvement in this study. Of these 40 subjects, 10 had *T. cruzi* in their blood, 10 exhibited cardiomyopathy or megacolon but no parasites were detected in their blood, 10 showed only positive antibody response against *T. cruzi* antigen by ELISA and 10 were healthy subjects as controls.

T. cruzi antigen

T. cruzi epimastigotes of RF isolate which was isolated in Paraguay from case of chagas' disease (Mimori *et al.*, 1992) were used to prepare the soluble antigen. Epimastigotes were washed 3 times by centrifugation in phosphate buffered saline (PBS) and then resuspended in PBS containing 1% TritonX-100 (Wako Chemical Co., Osaka, Japan), 1mM phenylmethylsulfonyl fluoride (Sigma Chemical Co., St Louis, MO) and 10% glycerol (Wako). The suspension was sonicated 5 times for 30 sec periods, followed by centrifugation at 10,000 x g for 1h. The supernatant was used as antigen.

ELISA

This assay was performed with an ELISA kit as previously reported (Maldonado *et al.*, 1990). Antibody titers were determined as the highest dilution of the test serum which gave an optical density ≥ 0.8 . Sera showing titers of over 1 : 20 were estimated to be antibody positive against *T. cruzi* antigen.

Gelatin-particles agglutination test (GPAT)

The GPAT was performed as previously described (Sato and Ryumon, 1990). Briefly, *T. cruzi* antigen (200 $\mu\text{g/ml}$) was conjugated to artificial gelatin particles (Fujirebio, Inc, Tokyo, Japan) treated with 10^{-5}M tannic acid. After conjugation of antigen, the gelatin particles were washed four times with 0.6% inactivated normal rabbit serum (NRS), then lyophilized and kept at 4°C until use. For estimation of agglutination titer, the lyophilized antigen-coated particles were resuspended to make a final 1% suspension in 0.6% NRS. One drop (25 μl) of the antigen-coated particles suspension was mixed in the U-bottomed wells with an equal volume of test serum in serial 2-fold dilutions. After settling at room temperature for 3h, agglutination patterns in the plates were read according to the results in the previous study (Sato and Ryumon, 1990). The antibody titer was determined as the highest serum dilution giving a positive agglutination pattern. Sera showing agglutination titers of over 1 : 16 were esti-

mated to be antibody positive against *T. cruzi* antigen.

RESULTS

The results of GPAT were compared with those of ELISA using sera of patients who have *T. cruzi* in their blood. As shown in Table 1, all patients were antibody positive by GPAT assessment and showed titers ranging from 1:16 to 1:1024. On the other hand, two acute cases with Romana's sign were shown to be antibody negative by ELISA assessment with a cutoff titer of 1:10.

GPAT and ELISA titers were then examined for 10 patients who were parasite negative in their blood but were found having cardiomyopathy or megacolon based on physical examination. The results, shown in Table 2, demonstrated that all patients were antibody-positive in both GPAT and ELISA. Antibody titers using GPAT were generally higher than those with ELISA.

Following the acute phase, most patients remain serologically positive, but asymptomatic, with the absence of demonstrative parasites in either their blood or tissues (Andrade and Andrade, 1979). Sera from persons having neither *T. cruzi* in their blood nor any symptoms, but which were antibody positive by ELISA, were examined to further estimate the antibody titer using GPAT. As shown in Table 3, all sera were antibody positive with GPAT and titers were higher than those determined by ELISA in 8 out of 10 samples.

Finally, sera samples from healthy subjects that were antibody negative to *T. cruzi* by ELISA were assessed by GPAT (Table 4). Ten sera used in this study were also antibody negative to *T. cruzi* antigen by GPAT. Furthermore, the gelatin particles used as a

Table 1 Comparison of antibody titers by GPAT and ELISA of sera of *T. cruzi* positive patients

Case No.	Titer	
	GPAT	ELISA
*1	1:128	1:10
*2	1:64	1:20
*3	1:32	1:10
*4	1:128	1:20
**5	1:128	1:80
**6	1:1024	1:160
***7	1:1024	1:80
***8	1:16	1:40
***9	1:32	1:40
***10	1:64	1:320

*Acute infection with Romana's sign

**Chronic infection with cardiomyopathy

***Chronic infection without lesions

Table 2 Comparison of antibody titers by GPAT and ELISA of sera of patients with lesions but who are parasite negative

Case No.	Titer	
	GPAT	ELISA
*1	1:512	1:320
*2	1:256	1:80
*3	1:128	1:80
*4	1:512	1:160
*5	1:4096	1:320
*6	1:256	1:40
*7	1:32	1:320
*8	1:64	1:40
**9	1:128	1:640
**10	1:1024	1:160

*Cardiomyopathy, **Megacolon

Table 3 Comparison of antibody titers by GPAT and ELISA of sera of subjects without lesions or parasites but who showed a positive antibody response to *T. cruzi* antigen by ELISA

Case No.	Titer	
	GPAT	ELISA
1	1:256	1:160
2	1:64	1:160
3	1:256	1:40
4	1:2048	1:160
5	1:128	1:80
6	1:32	1:40
7	1:512	1:320
8	1:128	1:320
9	1:512	1:80
10	1:512	1:40

Table 4 Comparison of antibody titers by GPAT and ELISA of sera from healthy subjects

Case No.	Titer	
	GPAT	ELISA
1	<1:16	<1:20
2	<1:16	<1:20
3	<1:16	<1:20
4	<1:16	<1:20
5	<1:16	<1:20
6	<1:16	<1:20
7	<1:16	<1:20
8	<1:16	<1:20
9	<1:16	<1:20
10	<1:16	<1:20

control did not react with any serum sample used in this experiment.

DISCUSSION

For diagnosis of Chagas' disease, either the *T. cruzi* parasite or a serological response to *T. cruzi* antigen must be obtained. Direct detection of *T. cruzi* in blood is possible during the acute phase but is difficult in patients with lower blood levels of the parasite. To overcome this problem, alternative procedures such as xenodiagnosis and hemoculture are used. Xenodiagnosis, however, is expensive, laborious, and limiting with respect to the length time to get results. In addition, the success rate is as low as 40% (Minter-Goedbloed *et al.*, 1978). In hemoculture, the blood sample is incubated in an appropriate medium for the growth of a potential parasite (Minter-Goedbloed, 1978) but a long cultivation period is needed and contamination can be a problem. A modern method using DNA probes allows for rapid and direct detection of the parasite in the blood (Gonzalez *et al.*, 1988). However, it is difficult to perform without specialized DNA probes and equipment.

In Chagas' disease, IgM antibody is first produced as a primary response and then IgG antibody appears 20-40 days after the onset of the acute phase of infection (Schmunis *et al.*, 1980). Many methods have been used to detect these antibodies, including complement fixation (Pereira *et al.*, 1980), hemagglutination (Camargo, 1971), indirect immunofluorescence (Cerisola *et al.*, 1970) and ELISA (Maldonado *et al.*, 1990). These techniques have various advantages and disadvantages based on requirements of special equipment, time required for testing and sensitivity.

GPAT was reported to be a simple and sensitive agglutination test for the screening of strongyloidiasis by Sato (1990). In this study, we confirmed the findings that GPAT is easy to perform and has a sensitivity comparable to ELISA and showed that this method could be used for serological diagnose of Chagas' disease. Furthermore, the antibody titer using GPAT was higher than that with ELISA in 76% of subjects who were shown to be antibody-positive to *T. cruzi* antigen. GPAT also requires no specialized equipment or facilities and results can be obtained within about 3h. In addition, the lyophilized antigen-sensitized carrier particles can be stored at 4°C for a long period, at least a year or more, without deterioration of the antigen. Moreover, the serum samples can be used without prior absorption by the gelatin particles because these non-

sensitized particles did not react with the 40 samples used in these experiments. The coloured gelatin particles were also found to be convenient for reading the agglutination pattern. Therefore, GPAT should prove to be useful for serodiagnosis of this disease, both in the laboratories as well as in the field.

In Latin America, distributions of *T. cruzi* and *Leishmania* infection often overlap. *T. cruzi* antigen has been shown to cross react with sera from leishmaniasis patients (Chaffee, 1956; Gam and Neva, 1977; Chiller *et al.*, 1990). In view of this, when parasitological examination is negative and symptoms are not characteristic of recent *T. cruzi* infection but the serological test is positive, there may exist the possibility of leishmaniasis or double infection. We examined ten serum samples from subjects without parasites or lesions. These sera were antibody positive to *T. cruzi* - antigen by GPAT and ELISA. However, it is still difficult to conclude whether these subjects had previous *T. cruzi* and/or *Leishmania*-infections. In the future, we will have to provide the specific antigen from *T. cruzi* and develop the specific sero-diagnosis for Chagas' disease.

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