Urinary Hydatid Antigen Detection by Coagglutination, a Cost-Effective and Rapid Test for Diagnosis of Cystic Echinococcosis in a Rural or Field Setting

P. T. RAVINDER,1 S. C. PARIJA,1* AND K. S. V. K. SUBBA RAO2

Departments of Microbiology1 and Cardio-Thoracic Surgery,2 Jawaharlal Institute of Postgraduate Medical Education and Research, Pondicherry, India

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We describe here coagglutination (Co-A), a rapid slide agglutination test for the detection of hydatid antigen in the urine for the diagnosis of cystic echinococcosis (CE). Paired urine and serum samples were collected from 16 patients with surgically confirmed CE, 10 patients with ultrasound-proven CE, 14 patients with clinically diagnosed CE, 24 patients with various parasitic diseases other than CE, and 25 healthy control subjects. Co-A detected excreted hydatid antigen in the concentrated urine of 7 of 16 (43.75%) surgically confirmed cases, 6 of 10 (60%) ultrasound-proven cases, and 8 of 14 (57.14%) clinically diagnosed cases of CE. A false-positive reaction was observed with 12.50% of control urine specimens from patients with parasitic diseases other than CE and 12% of urine samples from healthy controls. The circulating antigen was detected in the serum in 13 of 16 (81.25%) surgically confirmed cases, 6 of 10 (60%) ultrasound-proven cases, and 13 of 14 (92.86%) clinically diagnosed cases of CE. False-positive reactions were observed with three sera (12.5%) from controls with other parasitic diseases. The low sensitivity of Co-A for detection of antigen in the urine of a patient whose serum was positive for the antigen is possibly due to low levels of antigen in the urine. Unlike the collection of blood for serum, which is an invasive procedure and also requires technical expertise and disposable syringes, urine can be collected easily and frequently without causing any inconvenience to the patient. Urine as a clinical specimen alternative to serum would be immensely useful in the diagnosis of CE, particularly in a rural or field setting. In such situations as well as in poorly equipped laboratories, Co-A has the potential to be used as a simple, rapid, and economical slide agglutination test for detection of urinary hydatid antigen in the diagnosis of CE.

Human cystic echinococcosis (CE), caused by larvae (hydatid cysts) of the dog tapeworm Echinococcus granulosus, is a major infection having worldwide distribution and variable geographical incidence (17). Various assays have been developed and used for the detection of specific antibodies in serum with variable results (8). Antibody detection, however, has a major drawback in that this technique cannot readily be used to differentiate between recent and past infections (4, 6). This is due to the fact that the circulating hydatid antibodies persist even after clinical or parasitological cure (1, 7). Since circulating hydatid antigen is present in the serum only in active infections and the levels of these antigens in serum continue to decline after surgical removal or successful chemotherapy of the hydatid cysts, the detection of circulating antigen in the serum is suggested to be more useful than the detection of circulating antibodies in the serum for the diagnosis of active or recent CE (4, 13, 14).

Collection of blood for serum is an invasive procedure, and the procedure requires technical expertise and disposable syringes (9). If the method is not carried out under stringent conditions then it is associated with the risk of acquiring blood-borne infections such as hepatitis B virus and human immunodeficiency virus (HIV). Therefore, recently, there has been much interest in the use of urine as an alternative specimen to blood for the diagnosis of many parasitic infections (9).

Our group has developed for the first time a polyclonal rabbit hydatid antibody-based countercurrent immunoelectrophoresis designed to detect the presence of hydatid antigen excreted in the urine for the diagnosis of CE (12). This test performed well and could detect antigen in the urine collected from patients with surgically confirmed, ultrasound-proven, and clinically diagnosed cases of CE.

In the present study, we have standardized and evaluated prospectively the utility and cost of another simple and rapid slide agglutination test, coagglutination (Co-A), for detection of hydatid antigen in urine for diagnosis of CE. In our laboratory, the Co-A test was standardized and evaluated earlier for the detection of amoebic antigen in serum for the diagnosis of amoebic liver abscess (5), and for the detection of hydatid antigen in the serum (15) and antigen in the hydatid fluid (11) for the diagnosis of CE.

MATERIALS AND METHODS

Urine. Urine samples were collected from 89 patients attending the Jawaharlal Institute of Postgraduate Medical Education and Research Hospital, Pondicherry, India, as described previously (12). These included 16 specimens from patients with surgically proven CE, 10 specimens from patients with ultrasound-proven CE, and 14 specimens from patients with clinically diagnosed (presumptive) CE. Urine specimens also were collected from 24 CE-negative control subjects (patients with various parasitic diseases other than CE) and 25 healthy control subjects. Five milliliters of urine was collected from each patient in sterile glass vials using aseptic techniques, and specimens were stored at −20°C until use.

Concentration of urine. Part of each urine sample was concentrated by the method of ethanol precipitation as described previously (2). One milliliter of urine was mixed with 0.1 ml of a cold solution of 3 M sodium acetate, 0.1% bovine serum albumin, and 0.1% (wt/vol) sodium azide. To this solution, 1.5 ml of 96% (vol/vol) ethanol stored at −20°C was used. The mixture was blended in a vortex mixer and centrifuged at 10,000 × g for 10 min at 4°C. The supernatant was discarded, and the concentrated pellet of urine was resuspended in 0.1 ml of phosphate-buffered saline (PBS) (pH 7.2). Both unconcentrated and concen-
trated urine specimens from each patient were tested in parallel for hydatid antigen by Co-A.

**Hyperimmune antiserum.** Hyperimmune hydatid antiserum was raised in rabbits as per the procedure described by us earlier (15). The antibody titer of the antiserum was 1:1,024 as measured by the indirect hemagglutination (IHA) test. The antiserum was purified as per the method described by Gottstein (4). Briefly, 1 ml of cold serum was mixed with 1 ml of cold saline at pH 7. The serum-saline mixture (2 ml) was added dropwise to 2 ml of cold saturated ammonium sulfate (pH 7) with stirring for 30 min on ice and then centrifuging at 3,000 rpm at 0°C. The supernatant was discarded and the precipitate was suspended in 2 ml of saline, and the procedure was repeated until the supernatant was colorless. The final precipitate was suspended in 1 ml and dialyzed against PBS (pH 7.2) to remove all the residual ammonium sulfate. Titer of the purified antiserum was 1:2,048 by the IHA test.

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**Co-A.** The Co-A test was performed to detect hydatid antigen in the urine as per the procedure described herein. It consists of the following steps,

(i) **Preparation of bacterial cells.** *Staphylococcus aureus* (Cowans' strain I) containing protein A (SAPA) was used. The cells were prepared as per the method described by Shariff and Parija (15). Briefly, cells were grown on Mueller-Hinton agar at 37°C for 18 h and then were harvested, centrifuged at 3,000 rpm for 10 min, and the precipitate was suspended in 2 ml of saline, and the procedure was repeated until the supernatant was colorless. The final precipitate was suspended in 1 ml and dialyzed against PBS (pH 7.2) to remove all the residual ammonium sulfate. Titer of the purified antiserum was 1:2,048 by the IHA test.

(ii) **Sensitization of SAPA cells.** The SAPA cells were sensitized with purified hyperimmune hydatid antiserum immediately after their preparation. One milliliter of a 10% suspension of SAPA cells was added to 0.1 ml of specific antigen; these were mixed well and left at room temperature for 90 min; washed three times in PBS, pH 7.2; and resuspended to 10 million of cells in PBS, pH 7.2, at room temperature for 90 min; washed three times in PBS, pH 7.2; resuspended to 10 volumes of buffer containing 0.05% sodium azide; and heated for 5 min at 80°C. The antiserum was purified as per the method described by Gottstein (4). Briefly, 1 ml of cold serum was mixed with 1 ml of cold saline at pH 7. The serum-saline mixture (2 ml) was added dropwise to 2 ml of cold saturated ammonium sulfate (pH 7) with stirring for 30 min on ice and then centrifuging at 3,000 rpm at 0°C. The supernatant was discarded and the precipitate was suspended in 2 ml of saline, and the procedure was repeated until the supernatant was colorless. The final precipitate was suspended in 1 ml and dialyzed against PBS (pH 7.2) to remove all the residual ammonium sulfate. Titer of the purified antiserum was 1:2,048 by the IHA test.

**Co-A test.** The test was performed on a clean slide divided with a glass marking pen into two halves. A drop of test urine was placed on each half of the slide. An equal volume of 2% sensitized SAPA cell suspension was added to the urine on one half. The same volume of 2% suspension of unsensitized SAPA cells was added to the urine on the other half of the slide as cell control. The slide was then rotated manually and inspected. The urine specimen showing agglutination with the sensitized cells and not with the unsensitized cells was considered to be a positive result. Appropriate controls were examined in parallel with each test. Agglutination of serum with sensitized SAPA cells only and not with unsensitized SAPA cells was considered to be a positive result.

The urine and serum samples were tested in a single-blind manner. The sources of these specimens (whether they were from patients or control subjects) were not known to those performing the different tests with these specimens. The sensitivity, specificity, positive predictive value, negative predictive value, and prevalence of the tests evaluated in the study were calculated according to the method described elsewhere (3).

**RESULTS**

The results of the Co-A test with urine from the CE patients and control subjects are summarized in Table 1. The Co-A test detected hydatid antigen in the unconcentrated urine of four (25%) patients with surgically confirmed CE, two (20%) patients with ultrasound-proven CE, and three (21.42%) patients with clinically diagnosed cases of CE. The Co-A test also detected antigen in the unconcentrated urine of 2 (8.33)% CE-negative controls, and no antigen was detected in the urine of healthy controls.

Hydatid antigen was detected in the urine specimens concentrated by ethanol precipitation of 7 of 16 (43.75%) patients with surgically confirmed CE, 6 of 10 (60%) patients with ultrasound-proven CE, and 8 of 14 (57.14%) patients with clinically diagnosed cases of CE. The antigens were also detected in the urine of 3 of 24 (12.50%) CE-negative controls and 3 of 25 (12%) healthy controls. The Co-A test showed a sensitivity of 50%, specificity of 89.09%, positive predictive value of 81.25%, and negative predictive value of 79.03% (Table 2).

The hemagglutinating antibodies could be demonstrated by IHA in all groups of CE patients and controls (Table 3). An antibody titer of 1:128 in serum was considered to be diagnostic of CE. The Co-A test could also demonstrate hydatid antigen in the serum of the patients. Co-A detected circulating antigen in serum in 13 of 16 (81.25%) patients with surgically confirmed cases, 6 of 10 (60%) patients with ultrasound-proven cases, and 13 of 14 (92.86%) patients with clinically

<table>
<thead>
<tr>
<th>Urinary antigen by Co-A</th>
<th>Serum antigen by Co-A</th>
<th>Serum antibody by IHA</th>
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</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>50.0</td>
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<tr>
<td>Specificity</td>
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<tr>
<td>Prevalence</td>
<td>41.49</td>
<td>38.82</td>
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</tbody>
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**TABLE 3. Evaluation of Co-A and IHA tests in the diagnosis of CE**

The circulating hydatid antibodies in the serum were detected by the IHA test. This assay was carried out according to the procedure described by Parija and Rao (10). Double-aldehyde-stabilized red blood cells were sensitized with the optimal concentration of hydatid antigen. The hydatid antigen-sensitized double-aldehyde-stabilized cells were treated with serum to detect antibodies. The hemagglutination pattern of agglutinated red blood cells was noted only sensitized SAPA cells. In a negative test, no visible clumping was observed.

Serum. Serum samples were collected from all patients and control subjects.

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Serum. Serum samples were collected from all patients and control subjects.
diagnosed cases of CE. The antigen was also detected in sera of 3 of 24 negative controls. Tables 2 and 3 show the comparison of the IHA and Co-A tests for detection of serum antigen and urinary antigen in the diagnosis of CE.

DISCUSSION

CE is a major health problem worldwide, particularly in the sheep-rearing regions of Australia, South America, North Africa, Russia, and China (17). This disease is also an emerging disease in India, with incidences of CE being reported throughout the country (6, 16). The clinical diagnosis of CE is frequently difficult because of the variable site, size, and number of hydatid cysts in the infected host. Imaging methods such as ultrasound, computerized tomography, and recently, magnetic resonance imaging, are useful and of help in the demonstration and identification of hydatid cysts in most cases, but these imaging methods, nevertheless, are expensive and often not available in the area of endemicity for the disease. Therefore, development of a diagnostic method for CE that can be used under field conditions is a priority (7, 8).

The Co-A test is an indirect agglutination procedure performed on a glass slide that uses SAPA cells sensitized with hyperimmune hydatid antiserum. The binding of staphylococcal protein A to the Fe part of the immunoglobulin G predominately without affecting agglutinability of the bound immunoglobulin forms the basis of the assay (10). When the hydatid antibody-sensitized SAPA cells come in contact with the hydatid antigen in the urine specimen, the hydatid antibody and urinary hydatid antigen react, causing the sensitized SAPA cells to agglutinate.

The data presented in this study show that Co-A using a SAPA cell suspension coated with polyclonal hydatid antiserum is able to detect the presence of urinary hydatid antigen in specimens from patients with CE (Table 1). The Co-A test showed a sensitivity of 50% for the diagnosis of CE. The sensitivity was slightly more than that of countercurrent immunoelectrophoresis (47.5% sensitivity), which has previously been employed for detection of hydatid antigen in the urine (12). Co-A detected urinary hydatid antigen in the concentrated urine of 7 of 16 (43.75%) patients with surgically confirmed CE, 6 of 10 (60%) patients with ultrasound-proven CE, and 8 of 14 (57.14%) patients with clinically diagnosed CE. The sensitivity of Co-A for detection of antigen in urine was low compared to that of detection of antigen in the serum by the same test or detection of antibodies in the serum by IHA (Table 2). The low sensitivity of Co-A for detection of antigen in the urine of a patient whose serum was positive for the antigen by the Co-A test is possibly due to low levels of antigen in the urine. In such cases the use of a more sensitive assay such as enzyme-linked immunosorbent assay may be useful to detect the minute volumes of antigen in the urine that were not detected by Co-A. In hydatid serology, it is a fact that no single test, particularly not antibody-based immunoassays, is adequate to detect all cases of CE. Hence, to avoid false negatives, a combination of two or three antibody-based tests is suggested in order to detect the maximum number of cases of CE (6). Thus, when the urine Co-A result is negative but CE is strongly suspected, the detection of antigen in serum by Co-A or antibodies in serum by IHA would be a helpful supplement to the diagnosis of the disease.

The Co-A test for detecting urinary hydatid antigen offers many advantages. First, the technique is extremely simple, and even paramedical health personnel in a rural health center can perform this test on a microscopic glass slide. The test does not require any special equipment or technically trained or skilled manpower. This is important, particularly in view of the fact that CE is a disease which occurs predominantly in rural areas (8). The infected persons residing in these rural areas cannot afford to pay for high-technology approaches and expensive assays such as immunofluorescence, enzyme-linked immunosorbent assay, or radioimmunoassays. Second, the Co-A test is rapid; the result can be obtained within minutes of performing the test. Third, the use of SAPA cells as the principal Co-A reagent makes the test most economical for use in many of the laboratories in developing countries, such as India. The SAPA cells can be prepared with the help of a locally and commonly used simple centrifuge and once prepared can be stored for weeks in a refrigerator (4°C) without any loss of sensitivity (15). These factors make Co-A a simple, rapid, and economical test which can be used in a field or rural setting, a poorly equipped laboratory, or in a doctor’s clinic for on-the-spot detection of urinary hydatid antigen in the diagnosis of CE.

Urine, in comparison to blood, appears to be an ideal specimen because of its few advantages (9). First and foremost, urine can be collected easily and also frequently without causing any inconvenience to the patient. Second, as urine is collected by noninvasive procedure, the risk of blood-borne infections such as human immunodeficiency virus and hepatitis B virus, etc., associated with the collection of blood samples is avoided. Detection of urinary hydatid antigen might be particularly useful with the elderly, children, and other persons unwilling to provide blood for tests. It would also be immensely valuable in epidemiological studies with large-scale screening for CE.

REFERENCES