

RECENT TRENDS IN THE SERODIAGNOSIS OF HYDATID DISEASE

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Abstract. Hydatid disease caused by *Echinococcus granulosus* is a zoonotic infection of cosmopolitan distribution. As the clinical manifestations of hydatid disease in man are variable, the diagnosis of the condition presents complex problems for clinicians. Since the parasitic diagnosis of the disease is difficult, the specific diagnosis of the condition relies heavily on immunodiagnostic tests. The recent approach to the diagnosis of hydatid disease in man is primarily based on: (1) a combination of two or three more serological tests to diagnose the condition, as a single test fails to detect all the cases, (2) detection of circulating hydatid antigen (CAg) in the serum by enzyme-linked immunosorbent assay (ELISA) and other assays, as the antigen detection system is useful in monitoring post-surgical and chemotherapeutic evaluation of the cases as well as in the prognosis of the condition and (3) demonstration of *E. granulosus* antigen in the cystic fluid to establish the etiology of the hydatid cyst. Hydatid disease is essentially a disease of poor people residing in rural areas, hence there is need for a simple, economic diagnostic immunoassay for use at the field level or in a rural health center with inadequate facilities. Counter-current-immunoelectrophoresis (CIEF) and bacterial co-agglutination (Co-A), have been standardized and evaluated in this laboratory for the first time for detection of CAg in cases of hydatid disease at the field level and rural health center.

INTRODUCTION

This paper summarizes the studies on the immunodiagnosis of hydatid disease in Pondicherry in relation to the studies elsewhere. This paper also describes observations of the present author regarding the development of simple and rapid immunoassays for the detection of the circulating hydatid antigen in the serum at the field level and rural health centers.

Hydatid disease, caused by the larval stage of *Echinococcus granulosus*, is a chronic zoonotic disease having a worldwide distribution and variable geographical incidence (Williams *et al*, 1971). The diagnosis of hydatid disease in humans has presented complex problems for clinicians and laboratory workers. The clinical symptoms are invariably non-specific and associated with the pressure of hydatid cysts in the tissues of the host. Direct demonstration of the parasite or parasitic materials by aspiration biopsy of the hydatid cyst is inadvisable because of possible spillage resulting in the anaphylaxis and formation of secondary cysts. The specific diagnosis of the condition by usual methods, such as radiography, ultrasonography and com-

puterized axial tomography, used for the detection of space-occupying lesions is often difficult. This has led to widespread interest in the use of serological tests for the diagnosis of hydatid disease (Bhatia and Pathak, 1990). Immunodiagnostic tests play an important role in early diagnosis and treatment of the condition, thereby considerably reducing morbidity and mortality due to the disease. Serological tests are based primarily on the demonstration of circulating anti-echinococcal antibodies which occur frequently in established cystic hydatid infections (Kagan, 1968).

Even though India is not primarily a sheep rearing country, reports of hydatid disease have increased from different parts of India (Roy *et al*, 1970), such as Patna, Madras, Vellore, Gunur, Kurmool, Ahmedabad, Delhi, Amritsar and Jamnagar. It has also been reported from Assam, West Bengal, Orissa, and Himachal Pradesh, and areas in and around Pondicherry (Parija *et al*, 1987b). Since the first documented study of hydatid disease in Pondicherry (Parija *et al*, 1983) in early 1980, this laboratory has worked to develop simple and rapid serological tests to detect circulating antibodies and, more recently, antigens in the serum, to use at the field

level and in rural health centers for the diagnosis of hydatid disease.

DETECTION OF ANTIBODIES

Since the first attempt to devise a serological test by using a complement fixation test, a number of serological tests have been developed for the detection of circulating anti-echinococcal antibodies (Kagan, 1968) (Table 1). However, no single test provides complete sensitivity and specificity for hydatid disease. A combination of two or three tests, such as immunoelectrophoresis and double diffusion, indirect immuno-

fluorescence, indirect hemagglutination and latex agglutination, may be necessary to obtain reliable results (Chemtai *et al*, 1981). Many of these immunodiagnostic tests have been used with varying sensitivity and specificity for the demonstration of antibodies.

Hydatid disease in humans is essentially a disease of poor people who live in rural areas. Therefore, there is a need for a simple, economic immunoassay which would permit diagnosis of the disease in the field or in rural health centers with inadequate laboratory facilities. An attempt was made along this line in this laboratory, which is situated in an area endemic

Table 1
Immunodiagnostic tests in hydatid disease.

1. Complement fixation test	Ghedini 1906	Lower sensitivity (36-93%). Higher non-specificity (28%). Useful in post-op evaluation.
2. Hemagglutination test	Garabedian 1957	Sensitivity 66-100% (Kagan, 1968). Specific.
3. Latex agglutination test	Fischman 1960	Sensitivity 66-100%. (Kagan, 1968).
4. Indirect fluorescent antibody test	Fraga de Azevedo and Rombert 1965	Highly sensitive (100%). Employed to identify antibody immunoglobulin classes and to locate antigens in hydatid cyst material.
5. Immunoelectrophoresis test (IEP)	Capron <i>et al</i> 1967	Valuable in diagnosis of persistent or recurrent infection. Lower sensitivity, need for concentrating the serum and antigen, not suitable for mass screening as it requires large amounts of serum and antigen.
6. Counter current immunoelectrophoresis test	Castagnari and Sorice, 1971	Sensitive and specific. Shorter test time, small volumes of antigen and serum required.
7. Double diffusion test	Coltorti and Varela-Diaz 1978	Sensitive and specific.
8. ELISA	Farag 1975	As sensitive as IHA. Required small quantity of antigen, suitable for automation.
9. Radioimmunoassay	Musiani 1974	Higher sensitivity and specificity.
10. Lymphocyte transformation test	Miggiano 1966	Valuable in pulmonary hydatid disease where circulating antibodies are absent.

for hydatid disease. The indirect hemagglutination (IHA) test introduced by Garabedian *et al* (1957) for the serodiagnosis of hydatid disease, is inexpensive, easy to perform, and can be read without errors of subjectivity (Lewis *et al*, 1975). However, the conventional IHA in its present form is not suitable for use in a poorly-equipped rural health laboratory, primarily for the following two reasons: First, the procedure is time-consuming, requiring three successive steps of stabilization, tanning and sensitization of the red blood cells (RBCs) with hydatid cyst fluid antigen, each step being preceded and followed by the washing of RBCs with saline or buffer. Secondly, the shelf-life of the antigen-coated RBCs subjected to a single aldehyde stabilization and tanning treatment is short, even when stored at 4° C. Invariably the RBCs have to be prepared each time the test is performed. Hence, an effort was made by the authors to modify the IHA assay for routine application in less equipped laboratories and in those with less technical expertise, such as a rural health laboratory.

The IHA test could be a relatively simple and rapid diagnostic tool if it would be possible to preserve sensitized cells stored at 4°C for a longer period. In an earlier study (Parija and Ananthakrishnan, 1985), we evaluated the use of sheep RBCs, stabilized in various ways, in IHA tests on the sera of 21 surgically confirmed cases of hydatid disease and on control sera. Tests with double aldehyde stabilized cells (DAS) treated sequentially with pyruvic aldehyde, tannic acid and glutaraldehyde were more sensitive than tests with cells treated only with formaldehyde, glutaraldehyde or pyruvic aldehyde and subsequently tanned. The experience of these authors with filariasis (Parija *et al*, 1987a) and amebiasis (Parija *et al*, 1988) has also shown that the use of DAS cells greatly increased the sensitivity of the IHA and obviated the need for frequent preparation of sensitized cells.

Overnight delay in hemagglutination is another disadvantage. The use of sheep or human "O" RBCs, which are non-nucleated, requires an overnight incubation with test sera to obtain the definite settling pattern of hemagglutination. It makes IHA a time consuming procedure. To increase the speed of

the reaction, the nucleated cells from chicks, instead sheep, were evaluated in the IHA in hydatid disease (Parija *et al*, 1986). The test was performed with DAS chick cells on serum from 26 confirmed cases of hydatid disease and 45 control sera. The results were compared with those obtained in an IHA test with DAS sheep RBC using the same batch of sera; both tests were equally sensitive. The chick cells settled quickly and the result could be determined within 30–45 minutes. Heterophilic antigen was not a problem. This study also showed that without lyophilization, the hydatid antigen sensitized DAS prepared chick cells remain stable when stored at 4° C for 31 days. This study also established that sensitized DAS chick cells stored at 4°C for 31 days can be used as a ready-made reagent directly in the IHA test without affecting the sensitivity of the test. Results could be obtained within 60 to 90 minutes of receipt of the sera to be tested. Also the sensitized DAS RBCs may be prepared in a central laboratory and sent in a cold-chain to distant hospitals and laboratories to be used there for quick diagnosis of hydatid disease (Parija *et al*, 1986). This makes the IHA a simple and rapid procedure for serodiagnosis of hydatid disease at a less equipped laboratory.

Even though the IHA test is widely used, it is not as sensitive as an ELISA or RIA because some of the IgG bound to RBCs fails to cross-link the cells and hemagglutination does not occur. Cross-linking could be achieved by the use of an anti-immunoglobulin (Coombs *et al*, 1953). Alternatively, *Staphylococcus aureus*-bearing protein A can be used because this protein will bind IgG and subsequently agglutinate antigen sensitized RBCs. In this laboratory, we have developed a modification of the IHA, the protein A-IHA, which is as sensitive as ELISA for the diagnosis of hydatid disease (Parija and Rao, 1986). In the modified assay, the protein A-IHA, Cowan's strain of *S. aureus* which contains protein A was used to enhance hemagglutination of sensitized red cells. The test was performed in parallel with the IHA test on 31 sera from surgically confirmed cases of hydatid disease and on 45 sera from healthy blood donors. Use of *S. aureus* protein A enhanced sensitivity of the test and greatly increased the titers obtained with most of the

sera. Since Wood 46 strain of *S. aureus*, which lacks protein A, did not agglutinate sensitized DAS cells in the absence of antibody, co-hemagglutination appears to be mediated through protein A and occurs only in the presence of specific antibody. None of the sera from healthy blood donors showed false positive reactions. The sensitivity of this test compares well with the ELISA. Reagents for the protein A-IHA are inexpensive, easily available and stable. The sensitized RBCs and prepared suspension of *S. aureus* can be stored at 4° C for a longer period without loss of activity. The test is simple, inexpensive and does not require much technical skill; hence, it has the potential for wide application in the serodiagnosis of hydatid disease. The test has been modified further by the use of chick RBCs instead of human "O" RBCs, which makes the test a rapid procedure (Parija *et al*, 1987a).

As no single test provides complete sensitivity and specificity, the combination of two or three serological tests have been suggested to give most reliable results in the diagnosis of hydatid disease (Chemtai *et al*, 1981), but few efforts have been made to evaluate the possible use of Casoni's intradermal skin test along with a serological test. Our observation from a study of 29 surgically-confirmed cases of hydatid disease showed that the IHA test and Casoni's test, when used alone had a sensitivity of 75% and 47.3%, respectively, for the diagnosis of hydatid disease; however, when used in combination could establish the diagnosis in 27 (93.1%) of 29 cases. In addition, the IHA test was able to rule out 3 false positive reactions from Casoni's test. The later could diagnose 5 additional cases, which were negative by the IHA test. Thus, the combined use of Casoni's test and the IHA test is suggested for further evaluation in the reliable diagnosis of the hydatid disease (Parija and Rao, 1987).

DETECTION OF ANTIGEN

Many false negative reactions is the major problem associated with the immunodiagnostic methods detecting the circulating antibodies. The location, size and fertility of hydatid cysts in the tissues as well as low antibody responses in the human host (Chemtai *et al*, 1981) and the presence of immune complexes (Pini *et al*,

1983), are possible factors which contribute to the high incidence of false negatives in hydatid serology.

New avenues for the serodiagnosis of hydatid disease are needed using the approach of detecting circulating antigen in serum and other body fluids. Circulating antigens have been described in a variety of bacterial, viral, fungal and parasitic infections (Greenwood *et al*, 1971; Lalitha *et al*, 1989; Prince and Burke, 1970; Remington *et al*, 1972; Tselentis *et al*, 1981). In hydatid disease, the detection of circulating antigen (CAG) will be important in monitoring the disease, as it may more reliably reflect than antibody titers, the viability and quantity of the parasite in the host (Eckart and Gottstein, 1983). The CAG detection will also help in the diagnosis and post-surgical and chemotherapeutic followup of hydatid disease. The demonstration of CAG may also provide useful information on antibody negative hydatid cases and the status of the infection whether recent or past. The CAG was detected in mice by experimental infection with *Mesocestoides corti* and *E. multilocularis* (Alikhan and Siboo, 1983; Sogdandares-Bernal *et al*, 1981) and in rabbit by *Taenia pisiformis* (Craig, 1984). In human hydatid disease, immunodiffusion-in-gel was the first qualitative test to be employed for the detection of CAG in Greek patients by Tselentis *et al* (1981) and in Russian patients by Leikina *et al* (1982). Recently, a sensitive double antibody sandwich-ELISA was employed to detect CAG in the case of hydatid disease in Switzerland by Gottstein (1984). In another study, affinity purified rabbit and human antisera to antigen prepared from *E. granulosus* cysts was used to detect CAG in British and Kenyan patients by ELISA (Craig and Nelson, 1984). Thus, an ELISA is now available as a sensitive immunoassay to detect the antigen in human hydatid disease. The ELISA, though highly sensitive, is expensive and requires technical expertise, perishable reagents and is difficult to adapt in under equipped laboratories. A simple immunoassay to detect the circulating antigen in the hydatid disease is yet to be developed. Counter-current-immunoelectrophoresis (CIEF) and bacterial coagglutination (Co-A) are the widely used simple and rapid immunoassays for detecting antigens and anti-

bodies in a variety of parasitic, bacterial, fungal and viral infections. These two assays have high potential in detecting CAg in cases of hydatid disease. No reports are currently available suggesting the application of the two rapid and versatile immunoassays for the detection of hydatid antigen. In this laboratory CIEP and Co-A have been developed and evaluated in a study for the detection of circulating antigens in hydatid patients in Pondicherry. The Co-A was shown to be sensitive and specific. The test could detect CAg in the sera of surgically- and ultrasound-proven hydatid disease cases and clinically suspected hydatid disease cases. The test is simple, reliable and rapid; the result could be obtained within 30 to 45 minutes of receipt of the sera (Parija and Malini Shariff, in press). Similarly, the CIEP could detect the CAg in 55.55% surgically-proven and 100% ultrasound-proven hydatid disease cases. The test was highly specific, no false positive reactions were observed with sera from disease controls or healthy controls (Malini Shariff and Parija, in press). Therefore, the findings of the present study demonstrate that the Co-A and CIEP could be employed as single and rapid diagnostic immunoassays to detect circulating antigen in the hydatid disease at the field or in less than well-equipped laboratories; hence, they deserve additional evaluation for their wide application in the hydatid serology.

DETECTION OF ANTIGEN IN THE HYDATID FLUID

The diagnosis of hydatid cyst is confirmed by the demonstration of daughter cysts and scolices in the hydatid fluid, along with the histopathological evidence of the germinal layer of the cyst. The routine diagnosis is not always feasible by these methods in conditions where protoscolices have not been aspirated in the cyst fluid or are not present in the cyst. In such cases, it would be useful to identify the fluid as being of hydatid origin. An enzyme immunoassay to detect antigen in the hydatid cyst fluid, as an alternative to the direct parasitological methods has been reported recently (Craig *et al*, 1986). In this laboratory the CIEP as well as Co-A were successful in establishing the etiological diagnosis of 4 (80%) hydatid cysts by detecting *E. granulosus* antigen in the cyst fluid (Parija

and Malini Shariff, unpublished observation). The results of our study suggest that CIEP and Co-A tests can be employed as simple and rapid diagnostic procedures for the detection of antigen in hydatid fluid as an alternative to parasitological methods. We are now evaluating these two assays on additional cases of hydatid cysts for the demonstration of antigen in the hydatid fluid.

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