IMMUNODIAGNOSIS OF HYDATIDOSIS

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Hydatidosis is one of the serious helminthic zoonosis caused by the cystic stage of the tape worm, Echinococcus granulosus. The prevalence of hydatidosis is more in tropical and subtropical region and also in countries where cattle, sheep or hogs are extensively raised and utilized for human food, thus constituting a major public health and economic problem.

The disease causes considerable economic losses in food animals in developing countries including India due to the condemnation of liver, other organs and the whole carcass. Severe hydatidosis in animals' results in retarded growth, reduced quality and yield of milk, meat and wool. Clinical signs of hydatidosis are often non-specific in many cases. It also contributes to human infection. In India, various reports are available suggesting high endemicity of this disease. Though the infection is asymptomatic in many cases, the cysts situated in the vital organs interfere with the functions of affected organs with the danger of even fatality in certain cases. Hydatid cysts in the liver or spleen may present a malignant condition or an abscess. In the lungs cysts produce signs of an intrathoracic growth. Hydatid cysts in the bone cause rapid erosion of the bone leading to multiple fractures while those in the brain, heart, kidney or eye orbit causes serious symptoms and often are fatal. Cysts in the brain may cause epilepsy and blindness.

With the advent powerful drugs against cystic stages of cestodes, the prospect for their control by therapy is bright if diagnosed in the early stages. Immunological tests are important in the diagnosis of hydatidosis particularly, as there is invariably no direct parasitological evidence of infection and the clinical symptoms are not pathognomonic.

There are considerable differences among the various tests in both specificity and sensitivity. An optimum test should be specific with high sensitivity. Insensitive and nonspecific tests, including the Casoni intradermal test, the complement fixation test, the indirect haemagglutination test, and the latex agglutination test, have been replaced by the enzyme-linked immunosorbent assay (ELISA), the indirect immunofluorescence antibody test counterimmunoelectrophoresis (CIEP), and immunoblotting (IB) in routine laboratory application.

Casonis' skin test is an immediate hypersensitivity skin test performed by injecting 0.2 ml of sterile hydatid fluid intradermally. Development of a large wheal measuring 5 cm or more in diameter with multiple pseudopodia indicates a positive reaction. In most of the places, laboratory diagnosis is based mainly on Casoni's test. The main drawback of Casoni's test is lack of standardization of the antigen and high percentage of false positivity.

Immunodiagnostic tests are based on the detection of circulating antibodies as well as hydatid antigens.

DETECTION OF ANTIBODIES:

This method is based on detecting the circulating antibodies (mainly 1gG) by using hydatid antigens.
Antigens used for the diagnosis

Antibody responses in human hydatid infection were evaluated as early as 1965 by immunoelectrophoresis with sera from patients and Hydatid Cyst Fluid (HCF) of sheep origin as antigen (Chordi and Kagan, 1965). Further extensive studies have also focused on HCF antigens that are still considered an invaluable source of antigenic material for immunodiagnosis. Antigen prepared from human HCF was found to be unsuitable for diagnosis because it contains host proteins such as IgG. Sheep HCF obtained from fertile cysts has been used routinely to prepare and standardize antigen. Bovine HCF can be used as an alternative antigen source; indeed, it can improve diagnostic sensitivity. HCF of camel origin has also been used as antigen.

Antibody cross-reactivity with antigens from other parasites, notably other taeniid cestodes, is a major problem when using HCF antigens in immunodiagnosis

Researchers evaluated the immunoreactivity of these antigens with sera from patients with hydatid infection and came up with new techniques for the preparation of purified antigens It has been suggested that hydatid serology may be improved by the use of recombinant proteins, combining several defined antigens (including synthetic peptides) and the design of new E.granulosus-specific peptides. A novel DNA sequence from E. granulosus (termed EpC1) was isolated from a protoscolex (larval) cDNA library by immunoscreening, the sequence was expressed in bacteria, purified and the recombinant protein obtained was found to be of immense value in diagnosis of human hydatidosis (Jun Li et al, 2004).

Polysaccharide antigens from either the secretions produced during *in vitro* cultivation of E. granulosus protoscolices or from mouse hydatid cyst membranes by phenol extraction have been used to test sera from sheep (Jenkins and Rickard, 1986).

The lipoproteins antigen B (AgB) and antigen 5 (Arc 5), the major components of HCF, have received the most attention with regard to diagnosis. Along with HCF, they are the most widely used antigens in current assays for immunodiagnosis of the disease. Both antigens have been well characterized by immunoblotting and/or by immunoprecipitation of radio labeled antigen and SDS-PAGE.

Some of the diagnostic tests include;

Counter immunoelectrophoresis (CIEP)

The principle employed in this test is the formation of precipitation line when antigen and homologous antibody forms a complex.

Hydatid cyst fluid antigen and patient's test sera are electrophoresed in an agarose gel. Anodal wells are charged with patient's sera and cathodal wells with the hydatid antigen. Electrophoresis was run for 45 minutes and a line of precipitation between antigen and antibody well was considered as positive.

CIEP is simple to perform, cost effective, with reasonably high sensitivity and specificity. Localization of the cyst in different organs like the liver, lungs does not affect the sensitivity of the test while in other immunodiagnostic tests like indirect haemagglutination or indirect fluorescent antibody test, the sensitivity is reported to be more in hepatic than pulmonary cases.

Enzyme Linked Immunosorbent Assay (ELISA)

ELISA techniques using a variety of antigens have been applied to the immunodiagnosis of animal and human hydatidosis.

In this technique the antigen is attached to the solid phase to which test sera is applied. If homologous, the test sample gets attached to the solid phase. The antigen-antibody complex can be visualized by treating with antibody enzyme conjugate and a substrate. The colour developed is proportional to the antibody and antigen concentration.

Specific 1gG ELISA using AgB (antigen B-rich fraction) was the most sensitive test when compared with specific IgE ELISA and CIEP. Antigen capture ELISA, Sand witch Elisa, Dot ELISA are the various modifications used.

Western blotting (immunoblotting)

This is a highly sensitive and specific test for the diagnosis of human as well as animal hydatidosis. The basic technique is as follows:

The recombinant protein (serving as antigen) are transferred onto a nitrocellulose membrane using electrophoretic techniques. This nitrocellulose membrane is incubated with the human or animal test serum (Diluted 1 in 100 with 5% (w/v) skim milk in PBST) and, after incubation, treated with an antibody-enzyme conjugate. The location of the antibody-binding band is revealed by incubating the membrane with a substrate at room temperature for 15 min. The colour developing at the position of the antigenic protein band represents a positive reaction.

DETECTION OF ANTIGEN

Recently emphasis has been laid on detection of circulating hydatid antigen in the serum. CIEP, ELISA and bacterial-co agglutination are being now used to detect antigens.An ELISA test that detects the parasite products (coproantigens) in faeces has been developed. Coproantigens of Echinococcus can be readily detected in the feces of infected dogs and differentiated from those of the closely related Taenia species of tapeworms

commonly found infecting dogs (often in association with Echinococcus). The coporantigen ELISA is a very useful test because it requires no sophisticated equipment, is uncomplicated to perform and large numbers of specimens can be processed quickly. The test can be used under field conditions and shows great promise as a practical and safer means of monitoring the prevalence of dogs infected with E granulosus in hydatid control programs.

CONCLUSION

Immunodiagnosis is useful not only for primary diagnosis but also for follow-up of patients after surgical or pharmacological treatment. Serological tests plays a major role in the diagnosis and are mainly based on detecting the circulating serum antibodies. Recent serodiagnostic tests are based on detection of circulating hydatid antigen in the serum.

Advantages of antigen detection tests

1) It differentiates between recent and old infection as antigen disappears immediately after specific treatment.
2) It helps in evaluating pre-and post treatment and management of the cases as prognostic indicators.

Disadvantages of antibody detection tests

1) A single test shows low sensitivity. Hence, a combination of two or more tests are required to obtain reliable results.
2) They are not highly specific. Until recently it was believed that demonstration of antibodies against antigen 5 by immunodiffusion-ingel and counter immunoelectrophoresis, is highly specific for the disease, but now it has been demonstrated that "5-antigen" shows cross reactivity with sera from alveolar echi nococcosis and neurocysticercosis, and
3) They cannot differentiate between recent and old hydatid infections as the hydatid antibodies persist longer in the circulation.