Original Article

EVALUATION OF A dot-ELISA FOR THE SERODIAGNOSIS OF HUMAN HYDATID DISEASE

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ABSTRACT:
A dot-ELISA utilizing antigen-B from sheep hydatid fluid, dotted onto nitrocellulose filter discs was developed for the rapid diagnosis of human hydatidosis. 1µg of antigen per dot, serum dilution of 1:800, dilution conjugate of 1:1000 and 45 min incubation were found optimal.
Thirty four patients infected with hydatidosis, 32 cases with other parasitic diseases and 36 healthy subjects were included in the assay and were examined using dot-ELISA to detect antibody against the aforementioned parasite antigen. Sensitivity, specificity and positive and negative predictive values of the assay were calculated as 97.1%, 98.5%, 97.1% and 98.5% respectively.
No false positive reaction was observed when 36 sera healthy subjects were assayed. One case of cross-reactions was observed as for a serum infected with fasciolosis.
It was concluded that dot-ELISA is rapid, antigen and serum conservative as well as encompasses great importance to confirm clinical diagnosis either in the laboratory or in the filed.

KEYWORDS: dot-ELISA, hydatidosis, serodiagnosis.

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INTRODUCTION

Hydatidosis is one of the most important zoonotical diseases, which has a cosmopolitan distribution and prevalence worldwide[1] as well as in Iran[2]. In Iran, for example, hydatid disease is responsible for approximately 1% of admission to surgical wards, a figure which has increased remarkably recently due to increasing number of Afghan refugees residing in Iran[3].

Ingesting embryonated eggs through hands, food, drinks or material contaminated with parasite eggs infects humans, the larvae reach the blood and lymphatic circulation and are transported to the liver, lungs and other organs[1].

Diagnosis of hydatidosis besides image techniques (digital radiography, ultrasonography, computerized tomography and magnetic resonance imaging) and clinical findings relies on serological techniques[4]. Immunodiagnostic methods such as latex agglutination, indirect hemagglutination, complement fixation, indirect fluorescent antibody, precipitation tests, Western blotting and ELISA tests are currently used and in a bird eye view judgment, these assays confirm the diagnosis in 80% to 100% of hepatic hydatidosis cases and in 65% of pulmonary hydatidosis[4-6]. However there is a need for rapid and valid test to diagnose the

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disease. Unlike other currently performed tests for hydatidosis, dot-ELISA is said to be rapid, inexpensive and simple to do, as well as antigen and serum conservative[5]. In the present study and to evaluate these claims we challenged the validity of the test-using antigen-B of sheep hydatid fluid and human sera infected with hydatidosis.

MATERIALS AND METHODS

Antigen preparation

Hydatid preparations enriched in antigen-B was obtained from hydatid fluid as originally described by Ioppolo[7] and was modified by Orio[8]. Briefly, 100 ml hydatid fluid was dialyzed against 5 mM acetate buffer, pH 5 and centrifuged at 50000 g for 30 min. The precipitate was dissolved in 0.2 M phosphate buffer, pH 8.0. To remove any remaining host antibodies, 2.31 gr of ammonium sulphate was added and after one hour was centrifuged (3000g, for 30 min). The supernatant of salting out fluid boiled fir 15 min. and centrifuged at 50000g for 60 min. Finally the supernatant was collected and its protein content was measured. After filtration with 0.2 μl Millipore and adding nan3, it was stored at – 20°C for further utilization.

Sera

Thirty-four serum samples from patients infected with hydatidosis based on surgical operation confirmation were included in the test. Sera from others with different parasitic infections than hydatidosis including amoebiosis (n=5), fasciolosis (n=7), leishmaniosis (n=5), toxocariosis (n=5), and toxoplasmosis (n=5) were collected from the different laboratories in School of Public Health, Tehran University of Medical Sciences. Control serum samples were obtained from 36 volunteers at Tehran University of Medical Sciences, Iran. The human's ethics committee at the School of Public Health, Tehran, Iran, approved the study.

Dot-ELISA

Dot-ELISA was basically performed by the method as described by Zimmerman[9]. Briefly, 1 μg of antigen-B was dotted on nitrocellulose membrane disks and allowed to be dried thoroughly. The disks were placed into flat bottom micrometer plate well. Non-specific binding sites were blocked by addition 100 μl of tris buffer solution containing 0.5% Tween 20 (TBS/T) to each well. Blocking solution was then aspirated off and antigen disks were washed by shaking (three times, 10 min each) with 0.05% Tween 20 (Riedel de Haen, AG, Seelze, Hanover, Germany) in TBS (vol/vol). One hundred microliters of serum samples diluted 1:800 in TBS/T were added to each disk and incubated for 45 minutes at room temperature. The serum samples were removed and washings were conducted as described above. The washing solution was removed and 100 μl of horseradish peroxidase-labeled goat anti-human IgG conjugate (Sigma, Chemical Co) diluted in TBS/T were added to each well and incubated for 45 minutes at room temperature. The optimum dilution of the conjugate was found to be 1:1000 by block titration of two-fold dilutions of the conjugate. The conjugate was removed and other washings were conducted as mentioned before. One hundred microliters of the chromogen diamino benzidin tetrahydrocholoride (Sigma) was added to each well and incubated for 30 minutes at room temperature. The development of a deep brown colour dot on disks when compared with negative serum control was considered to be evidence of positivity. Colour development in negative controls was negligible or completely absent.

Statistical analysis

Standard diagnostic indices including sensitivity, specificity, positive and negative predictive values were calculated as described by Galen[10].

RESULTS

Optimization of dot-ELISA conditions

An optimum concentration of 1 ug of antigen-B of hydatid fluid per dot could detect

specific anti-hydatid IgG antibodies at 1:800 serum dilutions. Conjugate dilution of 1:1000 in TBS/T was found optimal when both antigen and sera were incubated for 45 min.

Application of dot-ELISA

Thirty-four patients infected with hydatidosis, 36 healthy normal controls and 32 cases with other parasitic diseases than hydatidosis were tested by dot-ELISA for antibodies against hydatid cyst antigen-B of 34 positive sera, 33(97%) gave a positive result to the dot-ELISA at 1:800 serum dilutions. Hence the sensitivity of the test was calculated as 97.1% (Fig. 1). All the control sera (36 cases) gave negative results.

The results of cross-reactions, using sera from the patients with other diseases are presented in Table-I. One case of cross-reaction was observed with a serum from patient with fasciolosis whereas serum samples from patients with amoebiosis, fasciolosis, leishmaniosis, toxocariosis and toxoplasmosis didn't cross-react (Table-I). In this regard the specificity of the assay was found to be 98.5% at the optimal serum dilution.

Other diagnostic parameters including positive and negative predictive values were detected as 97.1% and 98.5% respectively.

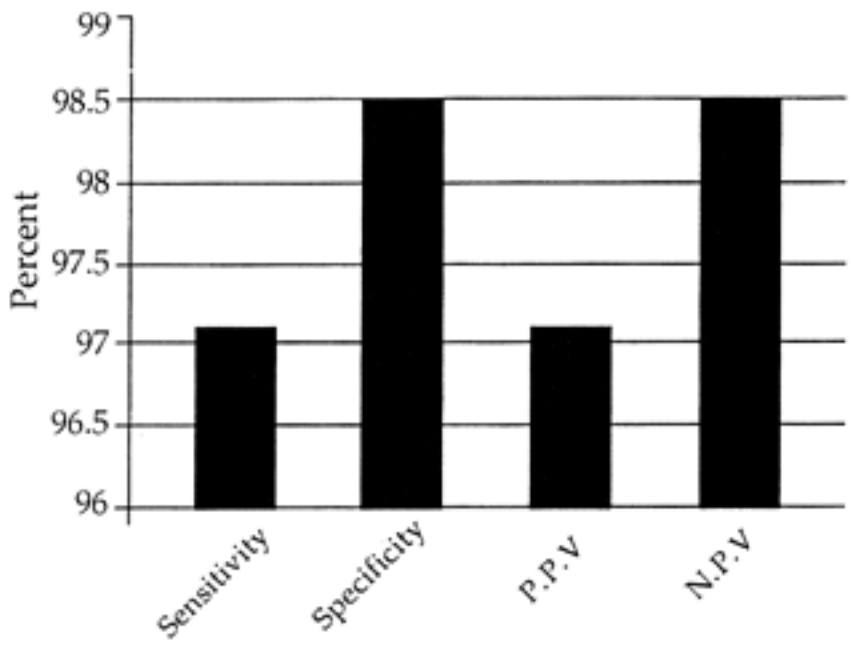

Figure 1: Diagnostic performance indices including sensitivity, specificity, positive predictive value (P.P.V.) and negative predictive value (N.P.V.) of the dot-ELISA for the diagnosis of human hydatidosis.

Table-I: Cross reactivity using sera from individuals with other parasitic diseases than hydatidosis and healthy control subjects

Groups	*Number*	*No. of cross-reaction*
Fasciolosis	12	1
Amoebiosis	5	-
Leishmaniosis	5	-
Toxocariosis	5	-
Toxoplasmosis	5	-
Healthy controls	36	-

DISCUSSION

Purification and preparation of suitable antigens is the basic and first step in serodiagnosis of hydatidosis[11]. Analysis of the hydatid cyst fluid, which has been used as a source of antigen, has revealed the presence of parasitic antigens shared with other helminthes, as well as host serum components[6,12]. The presence of such contaminants in the cyst fluid has limited its suitability for specific diagnosis of the disease. In order to overcome problems associated with the use of crude hydatid fluid in serological tests, various techniques have been developed for purification and separation of major hydatid fluid antigens[13,14]. In this regard we used of antigen-B from sheep hydatid cyst fluid in the test.

Compared with another standard diagnostic methods, dot-ELISA, offers several technical advantages which are as follows:[15-17]

- Stabling the antigen for long period after applying the antigens.
- Being reagent conservation of the assay.
- Enabling to test many samples in short period.
- If we consider that this method doesn't need to specific metric readings, the assay could be done in a field trial.
- Performing the incubation at room temperature.

The result of the present investigation demonstrated that dot-ELISA was a specific

mean of establishing serological diagnosis of human hydatidosis. Rogan[18] reported the sensitivity and specificity of this test as 94% and 90.3% respectively. In another survey, Sbihi[5], using aforementioned antigen, respectively reported the sensitivity and specificity of the test as 95% and 100%. Romia[17] reported these parameters for the assay as 88.9% and 96.9% in that order. We could demonstrate these parameters as 97% and 97.33% correspondingly, which shows higher degree than aforementioned results.

One case of cross-reactivity was observed between the test antigen and a serum sample from a patient with fasciolosis, suggesting that an antigen other than antigen-B in the hydatid cyst fluid shares epitope(s) with a fasciola immunogen and raises the possibility that this particular patients was also exposed to hydatidosis.

It was demonstrated that the dot-ELISA encompasses a great potential in the field and in the laboratory trials to diagnose human hydatidosis.

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