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Comparative Evaluation of Fast Enzyme Linked Immunosorbent Assay (Fast-ELISA) and Standard-ELISA For The Diagnosis Of Human Hydatidosis

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Abstract

Fast enzyme linked immunosorbent assay (Fast-ELISA) was compared with the standard ELISA for the diagnosis of human hydatidosis. Seventy serum samples including 30 from hydatidosis patients (surgically confirmed), healthy control individuals not infected with any parasitic diseases (n= 20) and from others with different parasitic infections including, toxocariosis (n=5), fasciolosis (n=5), trichostrongylosis (n=5), and strongyloidosis (n=5) were analysed for anti-hydatid IgG antibodies using sheep hydatid cyst fluid antigen. The sensitivity, specificity, positive and negative predictive values, as well as validity of the test were found as 96.7%, 95.2%, 93.7%, 97.5% and 96% for conventional ELISA, while these paramters for fast-ELISA were respectively as follows: 100%, 97.5%, 96.7%, 100% and 98.8%. Regarding standard-ELISA 3µg/ml of antigen, serum dilution of 1:500, conjugate dilution of 1:3000 and 30 min incubation were found optimal, while for fast-ELISA 3µg/ml of antigen, serum dilution of 1:125, conjugate dilution of 1:1000 and 5 min incubation were utilized. The present study indicates that fast ELISA can easily be performed in place of the standard ELISA for the serodiagnosis of human hydatidosis with the advantage of minimising consumed time and manpower hours. Moreover, this test can be utilized in screening tests to diagnos human hydatidosis.

Keywords: *Hydatidosis, Fast- ELISA, Standard-ELISA*

Introduction

Echinococcos granulosos is the causative agent of cystic hydatid disease or hydatidosis, a disease of a global distribution. Though the liver is the most frequently involved site, the cysts can develop in almost all organs of the body (1). The disease is established by the inadvertent ingestion of eggs via close contact with dogs, geophagy, contaminated vegetables and so on. The larvae reach the blood and lymphatic circulation and are transported to the liver, lungs, and other organs (1). Larval cysts or hydatids can be found in many tissues, most often in the liver, lung, mediastinum, peritoneum, and nearly every site of the body. Main clinical symptoms in humans include liver dysfunction, lung problems, ascites, abdominal pain, hepatomegaly, splenomegaly and central nervous system disorders.

Because there is not any production of the parasite into faeces, the laboratory diagnosis of hydatidosis mainly rests upon the detection of anti-hydatid antibodies in serum samples as well as clinical and radiological data.

Immunodiagnostic methods such as latex agglutination, indirect hemagglutination, complement fixation, indirect fluorescent antibody, precipitation tests, western blotting and ELISA tests, have been used in many laboratories and could confirm the diagnosis of hepatic hydatidosis in 80% to 100% and of pulmonary hydatidosis in 65% of the cases. (2-5).

Saving time, work forces, and reaching to a valuable diagnosis result as soon as possible,

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are the factors that necessitate evaluating a serological test like ELISA to be used in different laboratories. In this regard, the present study was conducted to evaluate a fast ELISA test in comparison with the conventional ELISA for diagnosing hydatidosis.

Materials and Methods

Sampling Seventy serum samples including thirty sera from hydatidosis patients (surgically confirmed), healthy control individuals not infected with any parasitic diseases (n=/20) and from others with different parasitic infections including, toxocariosis (n=5), fasciolosis (n=5), trichostrongylosis (n= 5), and strongyloidosis (n= 5) were analyzed for anti-hydatid IgG antibodies using sheep hydatid cyst fluid antigen.

Antigen preparation Antigen was prepared from the hydatid cysts obtained from naturally infected sheep, at the local slaughterhouse. Briefly, hydatid cyst fluid was drawn aseptically from the cysts and 5 mM phenylmethylsulphonyl fluoride was added. It was then centrifuged at 3000 g for 20 min to settle the protoscolices and the supernatant was taken as antigen and stored at -20 ºC until further use. The antigen protein concentration was estimated by the method of Bradford (6) with bovine serum albumin as a reference standard.

Study design Part 1: In this section, 100 microliters of hydatid crude antigen (3µg/ml) was dispensed into the wells of microtiter plates and were incubated at first for 1 h at 37 ºC followed by overnight at 4°C. The incubation periods between test steps were 30, 15, 10, and 5 min, respectively. Optimum dilutions of sera, antigen, and conjugate were 1: 500, 3ug/ml, and 1: 3000, respectively.

Part 2 (Fast-ELISA):This section represented the fast-ELISA. In this division, three plates were used. All plates were coated with 3µg/ml of hydatid antigen. Plate No.1(P1), was incubated at first for 1 h at 37 ºC followed by 24 h in refrigerator, plate No.2(P2) for 24 h at 37 ºC, and plate No.3(P3) for 2 h at 37ºC. The incubation period between test steps was 5min and optimum dilutions of sera, antigen and conjugate were 1:125, 3ug/ml and 1:1000, respectively.

Standard ELISA The immunodiagnostic assay was performed as previously described (6), with some modifications. Briefly, 100 microliters of hydatid crude antigen (3µg/ml) was dispensed into the wells of microtiter plates (Nuclon, Kamstrup, Roskilde, Denmark) and then incubated as the study design. Excess binding sites were blocked with 200µl of bovine serum albumin (2% diluted in PBS/ 0.1% Tween 20) and incubated for 30 min at 37 ºC. After the wells were washed three times with PBS/ Tween 20, 100 µl of a serum sample was added to each plate and incubated for 30 min at 37 ºC. Following another washing step, 100 µl of peroxidase-conjugated goat anti-human IgG was added to each well and the plates incubated for a further 30 min at 37 ºC. Following a final washing step 100 µl of O-phenylendiamine dihydrochloride (OPD) substrate (all from Sigma Chemical Co., Poole, Dorset, United Kingdom) was added to each well and the reaction stopped after 5 min by adding 50 µl of 12.5% H2S04. The optical density (OD) of the samples was measured at 492 nm using a Titerteck (Helsinki, Finland) multiscan ELISA plate reader. All assays were tested in triplicate and repeated twice.

Fast ELISA The technique used was the same as that of standard ELISA, with optimum dilutions of sera, antigen and conjugate as stated earlier. All the incubation periods at subsequent steps were reduced from 30 min to 5 min each.

Statistical analysis We used the mean plus 3.0 standard deviation OD value of the healthy group sera as the lower limit of positivity. The sensitivity and specificity were calculated using the method of Galen (7). Statistical analysis was carried out using SPSS for Windows, version 10.

Results

Table 1 and 2 show the sensitivity, specificity, positive and negative predictive values, as well as the cut off value for each studied parts.

Serum samples with an OD value greater than cut-off were considered positive for antihydatid antibodies by fast and standard ELISA methods. Hence,in part1 section, all 30 samples from surgically confirmed hydatidosis had positive antibody response while one case of strongyloidosis showed cross- reactivity with antihydatid antibodies.

In part two, one case of hydatidosis yielded negative response in test, while one case of strongyloidosis and one case of toxocariasis as for P1 and P2 plates, and the same cases plus one case of fasciolosis showed cross-reaction results.

Table 1: Sensitivity, specificity, positive and negative predictive values, as well as the cut off value for part 1 section

Incubation periods (min)	Sensitivity (%)	Specificity (%)	Pos. predictive value (%)	Neg. predictive value (%)	Cut-off
30	100	96.1	93.7	100	0.44
15	100	96.1	93.7	100	0.34
10	100	96.1	93.7	100	0.27
5	100	96.1	93.7	100	0.16

Table 2: Sensitivity, specificity, positive and negative predictive values, as well as the cut off value for part 2 section

Plates	Sensitivity (%)	Specificity (%)	Pos. predictive value (%)	Neg. predictive value (%)	Cut-off
P1	100	97.5	96.7	100	0.56
P2	100	97.5	96.7	100	0.51
P3	100	97.5	96.7	100	0.34

Discussion

Close relation with dogs for a long time embracing many diseases including human hydatidosis. Many people annually are infected with this infection,so finding and establishing a valuable diagnostic method, of course not aggressive, is of highly importance. Serological methods, particularly ELISA, have been evaluated by many researchers (8-10). Finding a technique to reduce as much as possible the sources of money and manpower as well as saving time and energy, necessitates establishing a new method or at least modifying the present methods. The use of fast ELISA technique has the potential to reduce the overall time from specimen collection to final identification, thereby, minimizing the reporting time and manpower hours (11, 12). Based on the Fast-ELISA features, the present study was designed to clarify the diagnostic issues.

A remarkable point in this study was the rate of cut-off. In part one section we noticed a decreasing rate of cut-off from 0.44 to 0.16, although the diagnostic parameters were the same for all plates. It is obvious that in comparison, a reasonable higher cut-off is more valuable than a lower one (13) i.e., the cut-off of 0.44 is more authentic to discriminate between patients and healthy people than 0.16. Hence, in conventional trial even, the diagnostic parameters were the same for all plates, but owing to abovementioned sentence, incubation period of 30 min is preferred to other periods. Meanwhile, in part two trial, i.e., fast-ELISA also the diagnostic parameters were the same and assuming that the former justification is correct, the plates P1 (1 h incubation after coating with antigen at 37 ºC followed by 24 h in refrigerator) and incubation periods of 5 min is recommended.

In a study conducted by Kaur et al., Fast enzyme linked immunosorbent assay (ELISA) was compared with the standard ELISA to diagnose hydatidosis (12). They reported the sensitivity and specificity as 82.3% and 100% by fast ELISA as well as 88.23% and 90.27% by standard ELISA, respectively. No cross-reac-

tions were observed with fast ELISA technique using samples from cysticercusis and amoebiasis patients as well as normal healthy controls. However, our study demonstrated only one case of strongyloidosis as cross-reaction with Fast-ELISA.

In conclusion, results of the study demonstrated that conventional ELISA technique could be replaced by fast-ELISA for the serodiagnosis of human hydatidosis. It is a fast, simple, valuable and convenient technique, which may serve as an useful adjunct to the clinical and radiological diagnosis.

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