Detection of anti-Echinococcus antibodies of human sera using ELISA

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Abstract:
To assess the anti-Echinococcal antibodies, human sera were collected from hydatid cyst patients and the antigen used was crude Hydatid fluid antigen (1.3mg/ml protein concentration) obtained from liver cyst of sheep, slaughtered in Rainwari abattoir (Kashmir). About 250 sera samples were collected from 90 cases of hydatidosis proven by surgical operation, 80 patients with diseases other than hydatidosis and 80 healthy cases. The results of indirect ELISA show 92.22% sensitivity and 98.75% specificity. In conclusion, the considerable immunoreactivity of the sera samples was detected in ELISA against the antigen preparation indicating their future usefulness as immunodiagnostic technique for Echinococcus infection in the humans.

Keywords:- Echinococcosis, ELISA, Diagnosis, Patients, Antigen.

INTRODUCTION

Hydatidosis caused by the larval stage of Echinococcus granulosus, is one of the most important zoonosis with world wide distribution, Nakao et al., (2010) and Mamishi et al., (2007). As it’s diagnosed by clinical symptoms and scanning alone is difficult and confusing, we have designed the present study to achieve a sensitive and simple diagnostic method for epidemiological studies.


Diagnosis of the condition is important not only for detection of cases but also for surveillance of the disease in the community and also for monitoring the impact of the control program for the disease in the area. Complement fixation test (CFT) was the first immunological test used for serodiagnosis of cyst hydatid disease. Since then, a wide variety of immunological tests have been developed for the detection of the hydatid disease antibodies and of late hydatid antigens in the serum. The hydatid antibased serological tests include indirect haemagglutination (IHA), indirect immunofluorescence (IFA), immunoelectrophoresis, counter-current immunoelectrophoresis, radioimmuno assay(RIA) and ELISA.

MATERIAL AND METHODS

Sera samples(250) were collected from 3 groups of the people including the 90 samples from the patients with hydatidosis proven by surgical operation from SKIMS Srinagar, 80 sera from the patients the disease other than hydatidosis and 80 sera samples from the healthy blood doners screened by a variety of standard imaging and paraclinical methods.

Hydatid infected livers of sheep slaughtered in Kashmir were collected and transported to the microbiology department of the SKIMS. The surfaces of the cysts were disinfected by iodine alcohol and cystic fluid was aspirated under sterile conditions. The suspension was centrifuged at (2000 x g at 4°C) for 1/2hr to separate protoscoleces and other solid agents. The supernatant was then dialysed through dialysing membrane (45mm pore size-Sigma company USA).

Indirect ELISA was performed as per the method described by Wittal et. al. (1986) with some modifications. ELISA plates were coated with the antigen 2µg per well diluted in coating buffer, (0.05m carbonate – bicarbonate buffer, pH9.6). The plates were incubated at 37°C for 1hr. Then the antigen were discarded and the wells were blocked with 200µl of blocking buffer (1% BSA Sigma USA). The plates were kept overnight at 4°C. Wells were washed four times by using washing buffer PBST, (PBS pH 7.2, containing TWEEN-20 , 0.05%). Test sera were diluted in PBS at the ratio 1 : 100, added into the wells and allowed for incubation at 37°C for 2 Hours. Again the plates were washed and 100µl of anti-ovine immunoglobulin conjugate (Horse radish Peroxidase, Sigma USA) was added at the dilutuoin of 1:5000.
The plates were incubated at 37°C. After 1 hr, the plates were washed with PBST and 100 µl substrate solution was added to each well. The plates were incubated in dark for 2 min. The reaction was stopped by adding 3N HCL. The optical density values were recorded by ELISA reader (Labsystem Multiskan) at 492 nm wavelength.

RESULTS AND DISCUSSION

The complete analysis of our data showed that the 90 cases of hydatidosis were of the age group of 25-80 years with the average of 48.11 (+1.8) years. Average age of 80 healthy cases was 28.25 (+0.85) years in the range of 18-46 years and in the 8th group 80 patients with the disease other than hydatidodises were of the age of 8-82 and the average of 36.86 (+2.3) years. Out of 90 samples, 83 were positive and 7 negative, whereas in 80 samples (non-hydatid patients), 2 were positive and 78 were negative and in healthy cases 80 samples were positive and 80 negative.

The sensitivity and the specificity values were 92.22% and 97.64% where as positive predictive value were 97.64% & 97.75%. ELISA test has been reported to be good from the point of sensitivity, specificity and repeatedly Maisonnave (1999) and Rogan et al (1991) have pointed a sensitivity and a specificity of 90.5% using dot ELISA with AgB as a field test for diagnosis of hydatid disease in Turkana region located in the north west of Kenya. The measurement of Hydatid specific IgG by the ELISA seems superior to and better than even the detection of hydatid specific IgE by the ELISA Afferni et al., (1984) with 5-A and B echinococcal antigens (Oriol 1971).


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REFERENCES:


Sheep Liver with Multiple Hydatid Cysts

Scolex of *E. granulosus* present in Hydatid cyst fluid (10x)
Sheep Liver with Mature Hydatid Cyst.

Scolex of *E. granulosus* present in cyst fluid (40x)

Scolex of *E. granulosus* present in cyst fluid (10x)


