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The Comparative Study of Some Immunological Technique and Diagnosis of Hydatidososis by Using Crude Ag of Hydatid Cyst

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Abstract

Echinococcosis is a cyclozoonotic infection of global distribution. It is one of the main forms of parasitic disease in farm animals that is caused by adult or larval stages of cestodes belonging to the genus Echinococcus, which use canines as definitive host and various herbivores or rodent as intermediate host, different species of Echinococcus cause different diseases in humans. The presented study was aimed to establish a noninvasive immunological test in Najaf Governorate. Of total of 80 clinical samples were collected from June to December 2021. The blood samples were taken from individuals infected with hydatid cyst disease and subjected to the initial identification was performed based on Imaging techniques like (X- ray, Ultrasound, MRI), surgical interventions. as well as Indirect immunofluorescence antibody test (IFA test) and enzyme-linked immunosorbent assay (ELISA test).This study noted that the results were as following , 91.25 %(73) of these cases were diagnosed firstly as hydatidosis by ELISA (IgG), 93.75 %(75) were positive by IFAT (total immunoglobulin) which considered as a confirmed hydatidosis cases and 85 %(68) were positive by using crude hydatid cyst fluid slide of E. granulosus by IFAT test.

Keywords

Hygaited cyst disease, crude hydatid cyst fluid, Non-invasive Technique, Immunofluorecent Technique (IFA).

Echinococcosis, commonly known as hydatid disease (hydatidosis), is a dangerous disease caused by the larval stage of cestode species relating to the genus Echinococcus. There are different forms of it, each of which has a unique adult appearance, host specificity, and pathogenicity [1]. Cystic echinococcosis is caused by the cestode, it is one of the major widely distributed zoonotic parasitic diseases in the Middle East and Arabic North African countries, Iran, Turkey, Jordan, Morocco, Libya, Tunisia, Algeria, Egypt and Iraq [2] According to Foodborne Disease Burden Epidemiology Reference Group (FERG) echinococcosis cause about 19 300 deaths annually over the world [3].It is considered one of the important parasitic diseases that infect livestock in Iraq and causes economic and public health significance. while alveolar Echinococcosis prevalence was rarely reported in [4][5][6]. In addition, there are four morphologically different cystic spp. which belong to this genus: Cystic echinococcosis is caused by E. granulosus, alveolar echinococcosis by E. multilocularis, and polycystic echinococcosis by E. vogeli and E. oligarthrus. The first

is the most prevalent of the four species. The second one is the most pathogenic, and the last two species are rare zoonotics [7][8]. and it is transmitted by blood, the hydatid Cysts larvae develop into (metacestode larvae), derived from parasites an encapsulated acellular layer is surrounded by a fibrous capsule composed of a germ layer with an inner core.Brood capsules and primary particles bud from the germinal membrane range of Intermediate host species depend on infection E. granulosus strain and the larval stage of the tapeworm Echinococcus [9]. Also, the fluid of hydatid cyst has been recognized as a complex mixture of substances, some attributed to the host, others to the parasite metabolizing in the cyst. Various immunological testing has revealed the fluid to be reactive as well. Variations in potency of different cyst fluid antigens have been reported. Some investigators have associated the variability in antigenicity with the animal host, suggesting, for example, that bovine hydatid fluid is less antigenic than human or porcine hydatid fluids. Others, finding as much variability from cyst to cyst [10]. A complementary diagnostic method can detect hydatid cyst disease, and it may also monitor patients after surgical or pharmacological treatment[11]. Asymptomatic populations can benefit from early diagnosis with it as it has proved to be effective[12].

This study conducted to establish a non-invasive test (immunological test) of hydatid cyst disease depending on (crude hydatid cyst fluid) by using an indirect immunoflorecent test,and was compared with the following standardized techniques: the indirect immunofluorescent antibody test (IFAT) and the enzyme-linked immunosorbent assay (ELISA).

Materials and methods

Manufacturing of slides of ready Indirect immunofluorescence kit.

fluid collection

Sheep hepatic Hydatid fertile sac were collected from Najaf slaughterhouse, placed in clean containers and transported to laboratory of microbiology department of medical faculty of Jabber Ibn Hayan university.

According to AL-Omoran et al.2 (1995) method, the surface of hydaitd sac was sterilized by ethyl alcohol (70%), then the fluid was drawn by deposable plastic syringes (10-20 cm3). The Hydatid cyst fluid was collected in the sterile tubes.

Serum collection

1-By using deposable plastic syringes (3-5) cm3, blood drawn from each patient infected with Hydatid cyst disease.)

2- Allow the blood to clot for 10-20 minutes at room temperature. Centrifuge (at 2000-3000 RPM) for 20 minutes. Collect the supernatants (serum) carefully and store immediately at - 20°C until to use.

Preparation of the slides by using papanicolaou method

1.smear was made from crude Hydatid cyst fluid by adding one drop on slide to each patient infected with Hydatid cyst disease.

2. The slides were left to dry then fixed in equal parts of 95%ethonol and ether for 5-15 minutes.

3. All slides were kept in the refrigerator until to use.

4. All sera had been used in indirect immunoflorescent procedure. Indirect immunoflorescent procedure was used with fixed smear.

Manufacturing of slides of Anti- crude Hydatid cyst fluid of Echinococcus granulosus of ready indirect immunofluorescence kit.

1.smear was made from crude Hydatid cyst fluid by adding one drop on slide to each patient infected with Hydatid cyst disease.

2. The slides were left to dry then fixed in equal parts of 95%ethonol and ether for 5-15 minutes.

3. All slides were kept in the refrigerator until to use.

4. All sera had been used in indirect immunoflorescent procedure. Indirect immunoflorescent procedure was used with fixed smear.

5.All sera have been used in indirect immunoflourescent procedure.

6.Indirect immunofloresent procedure was used with slides.

Results

Sero-positive cases of hydatid cyst by IFA tests

All structure of frozen section of E.granulosus. The

same style must ultimately be gained as for the positive control serum. Which recorded 93.75 % of cases. (Table 1 &Figure 1)

Control (16)		Patients (80)		Test
negative	positive	negative	positive	IFA
100%	Zero	6.25 % (5)	93.75 % (75)	

Table 1: The positive control serum.

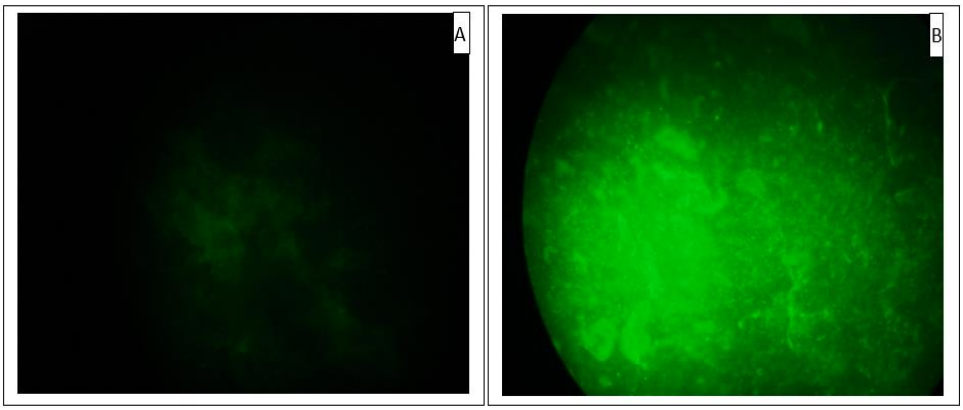

Figure (1): (A) positive case of frozen section of E. granulosus by IFA test. (B) Negative case of frozen section of E.granulosus by IFA test (×40).Sero-positive cases of ELISA Tests.

The percentage of ELISA IgG of patients infected with hydatid cyst disease was 91.25% of cases. (Table 2)

Table 2: ELISA IgG of patients infected.

Control (16)		Patients (80)		Test
negative	positive	negative	positive	ELISA
100%	Zero	8.75 % (7)	91.25 % (73)	

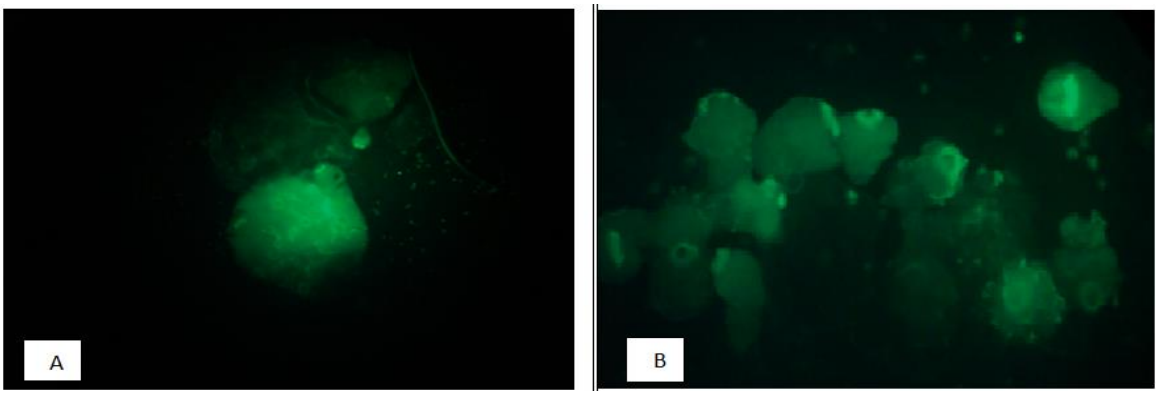

Figure (2): (A)positive case of crude hydatid cyst fluid slide of E. granulosus by IFAT test (×40).

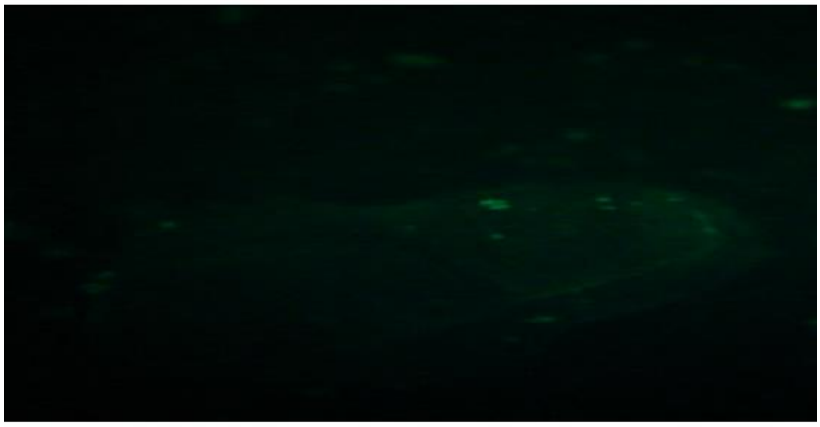

Figure (2): Negative case of crude hydatid cyst fluid slide of E.granulosus by IFA test (×40)

Sero-positive cases of crude hyadatid cyst fluid slides by IFA Tests.

The crude hyadatid cyst fluid slides gave a high percent (85 %) of patients, where 68 cases of total (80) haydatid cyst patients showed positive, but no case (0%) of 16 healthy individual showed positive. (Total3 & figure 2).

Table 3: The percentage of positive cases of hyadatid cyst fluid slides by IFA test as noninvasive investigation.

Control (16)		Patients (80)		Test
negative	positive	negative	positive	IFA crude hyadatid cyst fluid slides
100%	Zero	15 % (12)	85 % (68)	

The results of different tests of hydatid cyst disese. The highest percentage recorded (93.75 %) of Hydatid cyst disease was by IFA test as positive cases.

Table (4): Sero positive and negative by different cases

Control 16		Patients 80		Test
Negative	Positive	Negative	Positive	IFA
100% (16)	0.0 % (Zero)	6.25 % (5)	93.75 % (75)	
100% (16)	0.0 % (Zero)	8.75 % (7)	91.26 % (73)	ELISA
100% (16)	0.0 % (Zero)	15 % (12)	85 % (68)	IFA crude Hydatid cyst fluid slides

Discussion

The crude hydatid cyst fluid of E.granulosus were used as whole antigen substrates after fixation on the slides for the indirect IFA test which made manually. The percentages of positive cases were 85% from all infected cases. The results came agree with study of wattle et al. ([1], who reported that, immunofluorecent test was specific for whole scolex antigen, that gave four plus fluorescence of scolex with a positive serum. Sanchez et al [(9).]. used monospecific rabbit antisera obtained through experiential immunization with formerly refined proteins, in the ultrastructural localization of [(2)]. Hydatid fluid antigens; antigen B and antigen 5, in board capsules and protoscolices of E. granulosus of human source. response was apparent in the connective region of the germinal membrane and in the parenchyma of the protoscolices. Davis et al3 (1978) revealed that both antigens appear to originate in the protoscolices and/or the germinal membrane of the cyst and board capsules[(3)].

The result of the current study agrees with many studies using ELISA native Ag5, which have evidenced diagnostic sensitivities and specificities ranging between 50-54% and 89-92% respectively [11.5.] Also, these results agree with a study showed that the performances of the serological tests were highly sensitivities and may reach

to 90% when using somatic extracts from protoscolices and adults [10,12.18]

The premier immunological reaction of E. granulosus in the definitive host is directed against infective protoscolices and later, against the adult parasite. Because of this, somatic extracts from protoscolices and adults have been the most suitable source of antigens in the immune detection of the infection in dogs and other canids [4,12.]

The present study agree with studies which demonstrated that antigenic components of 27 and 94 KDa from crude protoscolex extracts were specifically specified by 95%and 62% of dog sera experimentally stomached with E. granulosus respectively [4,12]. Interpretation of these deferent results may be attributed to the fact that the E[13,14.] granulosus larva synthesize a lipoprotein known as antigen B (AgB) in the tegmental cells of the protoscolices more than the laminated and germinal layer of the brood capsules before start secreted in the hydatid cyst fluid [(1)].

Extensive studies are required to investigate the epidemiology of the disease in al Najaf and also all-around Iraq to constitute a control program as well as in the other countries especially in the Middle East such as turkey.[16,17.] In this regard, serious consideration should be given.

Suitable measures should be implemented. There should be a national or regional program to control hydatids.

References

M. Nakao, A. Lavikainen, T. Yanagida, and A. Ito,"Phylogenetic systematics of the genus Echinococcus (Cestoda: Taeniidae)," Int. J. Parasitol., vol. 43, no. 12–13, pp. 1017–1029, 2013, doi: 10.1016/j.ijpara.2013.06.002.

[S. M. Sadjjadi,"Present situation of echinococcosis in the Middle East and Arabic North Africa," Parasitol. Int., vol. 55, no. SUPPL., pp. 197–202, 2006, doi: 10.1016/j.parint.2005.11.030.

L. A. Kadir, M., & Saaid,"The effect of albendazole and mebendazole on Echinococcus granulosus protoscolicies.," Med. J. Tikrit Univ. [The], 3(2), 115-120., vol. 21, no. 3, pp. 295–316, 1997.

M. B. Rokni,"Echinococcosis/hydatidosis in Iran," Iran. J. Parasitol., vol. 4, no. 2, pp. 1–16, 2009.

A. E. Alsaimary, H. M. Abdulnbi, A. Laibi, and A. R. Jwad,"The occurrence of Helicobacter pylori in hydatid liver disease," Asian Pac. J. Trop. Biomed., vol. 2, no. 3, pp. 233–234, 2012, doi: 10.1016/S2221-1691(12)60048-3.

R. Morar and C. Feldman,"Pulmonary echinococcosis," Eur. Respir. J., vol. 21, no. 6, pp. 1069–1077, 2003, doi: 10.1183/09031936.03.00108403.

B. Boufana, J. Qiu, X. Chen, C. M. Budke, M. Campos-Ponce, and P. S. Craig,"First report of Echinococcus shiquicus in dogs from eastern Qinghai-Tibet plateau region, China," Acta Trop., vol. 127, no. 1, pp. 21–24, 2013, doi: 10.1016/j.actatropica.2013.02.019.

D. Poretti et al.,"Differential immunodiagnosis between cystic hydatid disease and other cross-reactive pathologies," Am. J. Trop. Med. Hyg., vol. 60, no. 2, pp. 193–198, 1999, doi: 10.4269/ajtmh.1999.60.193.

I. G. Kagan and M. Agosin,"Echinococcus antigens.," Bull. World Health Organ., vol. 39, no. 1, pp. 13–24, 1968.

F. SÁNCHEZ et al.,"Ultrastructural localization of major hydatid fluid antigens in brood capsules and protoscoleces of Echinococcus granulosus of human origin," Parasite Immunol., vol. 15, no. 8, pp. 441–447, 1993, doi: 10.1111/j.1365-3024.1993.tb00629.x.

G. González-Sapienza, C. Lorenzo, and A. Nieto,"Improved immunodiagnosis of cystic hydatid disease by using a synthetic peptide with higher diagnostic value than that of its parent protein, Echinococcus granulosus antigen B," J. Clin. Microbiol., vol. 38, no. 11, pp. 3979–3983, 2000, doi: 10.1128/jcm.38.11.3979-3983.2000.

A. C. Arend, A. Zaha, F. J. Ayala, and K. L. Haag,"The Echinococcus granulosus antigen B shows a high degree of genetic variability," Exp. Parasitol., vol. 108, no. 1–2, pp. 76–80, 2004, doi: 10.1016/j.exppara.2004.07.009.

C. Wattal, N. Malla, I. A. Khan, and S. C. Agarwal,"Comparative evaluation of enzyme-linked immunosorbent assay for the diagnosis of pulmonary echinococcosis," J. Clin. Microbiol., vol. 24, no. 1, pp. 41–46, 1986, doi: 10.1128/jcm.24.1.41-46.1986.

"Al-Omoran, A. H., Emman, G., & Altaif, K. I. (1995). Immunoprophylaxis with Nocardia asteroids cell wall extracts of experimental hydatidosis in Balb/c mice. Dirasat Agricultural Sciences, 3, 189- 198.," p. 1995, 1995.

C. Davies, M. D. Rickard, J. D. Smyth, and D. T. Bout,"Ultrastructural immunocytochemical localization of two hydatid fluid antigens (antigen 5 and antigen B) in the brood capsules and protoscoleces of ovine and equine Echinococcus granulosus and E. multilocularis," Parasitology, vol. 77, no. 2, pp. 143–152, 1978, doi: 10.1017/S0031182000049349.

M. Barbieri, V. Fernández, G. González, V. M. Luaces, and A. Nieto,"Diagnostic evaluation of a synthetic peptide derived from a novel antigen B subunit as related to other available peptides and native antigens used for serology of cystic hydatidosis," Parasite Immunol., vol. 20, no. 2, pp. 51–61, 1998, doi: 10.1046/j.1365-3024.1998.00117.x.

D. J. Jenkins, R. B. Gasser, E. Zeyhle, T. Romig, and C. N. L. Macpherson,"Assessment of a serological test for the detection of Echinococcus granulosus infection in dogs in Kenya," Acta Trop., vol. 47, no. 4, pp. 245–248, 1990, doi: 10.1016/0001-706X(90)90016-S.

R. B. Gasser et al.,"Immunological assessment of exposure to Echinococcus granulosus in a rural dog population in Uruguay," Acta Trop., vol. 58, no. 3–4, pp. 179–185, 1994, doi: 10.1016/0001-706X(94)90012-4.

L. Golassa, T. Abebe, and A. Hailu,"Evaluation of crude hydatid cyst fluid antigens for the serological diagnosis of hydatidosis in cattle.," J. Helminthol., vol. 85, no. 1, pp. 100–108, 2011, doi: 10.1017/S0022149X10000349.

R. B. Gasser, D. J. Jenkins, D. D. Heath, and S. B. Lawrence,"Use of Echinococcus granulosus worm antigens for immunodiagnosis of E. granulosus infection in dogs," Vet. Parasitol., vol. 45, no. 1–2, pp. 89–100, 1992, doi: 10.1016/0304-4017(92)90030-D.

A. Ito et al.,"Control of echinococcosis and cysticercosis: A public health challenge to international cooperation in China," Acta Trop., vol. 86, no. 1, pp. 3–17, 2003, doi: 10.1016/S0001-706X(02)00269-3.

H. O. Lobel and I. G. Kagan,"Seroepidemiology of parasitic diseases.," Annu. Rev. Microbiol., vol. 32, no. 33, pp. 329–347, 1978, doi: 10.1146/annurev.mi.32.100178.001553.

"Anderson FL, Ouhelli H, Kachani M. Compendium on Cystic Echinococcosis in Africa and in Middle Eastern Countries with Special Reference to Morocco. Provo (UT): Brigham Young University Press; 1997.," p. 1997, 1997.

M. A. Shambesh, P. S. Craig, C. N. L. Macpherson, M. T. Rogan, A. M. Gusbi, and E. F. Echtuish,"An extensive ultrasound and serologic

study to investigate the prevalence of human cystic echinococcosis in Northern Libya," Am. J. Trop. Med. Hyg., vol. 60, no. 3, pp. 462–468, 1999, doi: 10.4269/ajtmh.1999.60.462.