

EVALUATION OF ELISA AND IHA IN SEROLOGICAL DIAGNOSIS OF PROVEN CASES OF HUMAN HYDATIDOSIS

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Abstract

Cystic hydatid disease (Hydatidosis) is the most serious tape-worm infection prevalent in the cattle and sheep raising area of the world. Hydatidosis in man (as an accidental host) is caused by infection with the ova containing larval stage of *Echinococcus granulosus*. In the last decade, different techniques have been employed for sero-diagnosis of hydatidosis; as IHA, IFA, ELISA, CCLE (Counter Current Immuno-electrophoresis). This paper evaluated the validity of ELISA and IHA. Since whole hydatid cyst fluid was used as a source of antigen for serodiagnosis. Thirty surgical and pathological hydatidosis proven patients were examined. The sensitivity and specificity of ELISA were 96.7% & 97.5% respectively, and that of IHA were 86.7%, & 95% respectively.

Key words: Human hydatidosis, ELISA, IHA, Cross reactivity.

Introduction

Hydatid disease or echinococcosis is a zoonotic disease caused by larval stage of *Echinococcus* species (Matossian *et al.*, 1977). The word 'hydatid' of Greek origin, literally means a 'watery vesicle' (Milicevic, 1994).

Till now, hydatidosis remains endemic in many countries, most notably the Mediterranean region,

the Middle East, South America, New Zealand, and Australia due to the close association between man and both farm animals and dogs (Kuzuzcu *et al.*, 2004).

In Egypt, many authors (Cahill *et al.*, 1965; Romia *et al.*, 1992; Abdel Rahman *et al.*, 1992; Mazyad *et al.*, 1998; 1999; Ramzy *et al.*, 1999; Abbas *et al.*, 2006; El-Shazly *et al.*, 2007; Haridy *et al.*, 1998; 2000; 2007; 2008a,b; Ramadan and El Damaty,

2000) reported human and animal hydatidosis. Man is a blind end host when becomes accidentally infected (Wilson, 1991).

Immunodiagnosis was useful not only for primary diagnosis but also for follow-up after surgical or therapeutic treatment. Detection of circulating *E. granulosus* antigens in sera was less sensitive than antibody detection, which remains the method of choice. There are considerable differences among the various tests in both specificity and sensitivity. An optimum test should be specific with high sensitivity (Zhang *et al.*, 2003).

Subjects, Material and Methods

Three patients groups were used.

GI: included 30 hydatidosis patients with no other parasitic infections. GII: included 30 cases infected with other parasites as *S. mansoni*, *Fasciola*, *H. nana*, *E. histolytica* and *Giardia lamblia*. Each group of patients included six from each infection. GIII: included 10 healthy parasite-free cases as controls.

Serum samples were collected and storage at -20°C until use for detection of antibodies against *E. granulosus* by using IHA and ELISA.

The IHA was done by using Echinococcosis kit (Fumouze Diagnostics, Levallois-Perret, France) after the manufacturer's instruction. The ELISA IgG was done by (Bordier Affinity Products SA; Switzerland; Article No 9350).

Results

Table 1: Diagnosis of 30 proven hydatidosis patients by ELISA and IHA.

Sera	ELISA		IHA	
	Positive	Negative	Positive	Negative
	No(%)	No(%)	No(%)	No(%)
Positive (n= 30)	29(96.7)	1(3.3)	26(86.7)	4(13.3)
Other parasites (n=30)	1(3.3)	29(96.7)	2(6.7)	28(93.3)
No. negative (n= 10)	0	10(100)	0	10(100)

Table 2: ELISA and IHA cross-reactions with other human parasites.

Parasites	No. tested	ELISA	IHA
		Number of positive reaction	
<i>Fasciola</i> cases	6	1	1
<i>S. mansoni</i> cases	6	-	1
<i>Hymenolepis nana</i> cases	6	-	-
<i>E. histolytica</i> cases	6	-	-
<i>Giardia lamblia</i> cases	6	-	-
Total	30	1	2
Cross-reaction (%)		(3.3%)	(6.7%)

Table 3: Parameters of IHA and ELISA in diagnosis of human hydatidosis.

Test	Cut-off value	Sensitivity	Specificity	J index	Accuracy	Kappa	PPV	NPV
ELISA	0.394	96.7	97.5	94.17	97.14	0.942	96.67	97.5
IHA	320	86.67	95	81.67	91.43	0.824	92.86	90.48

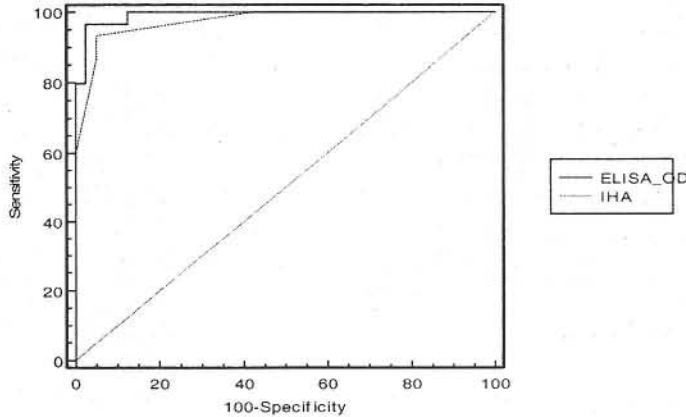


Fig. 1: Comparison of ROC curves of ELISA and IHA using all controls.

Discussion

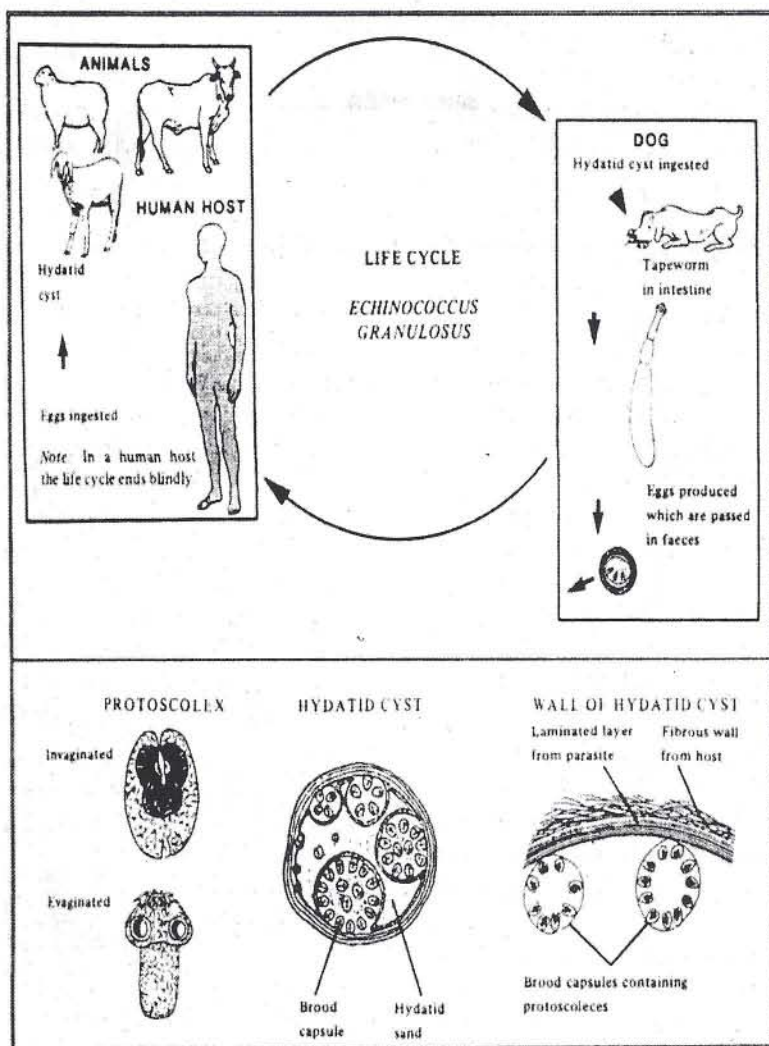
In the present study, results of IHA revealed two negative cases with titer 1:80, two cases equivocal with titer 1:160 and 26 cases gave significant reaction. The titers were as followed 8 cases were positive at 1: 320, 6 cases were positive at 1: 640, 7 cases were positive at 1: 1280, 5 cases were positive at 1: 2560. The results of IHA according to age showed that the largest number of cases were positive in the age group >40 years (15/26) cases (57.7%). As regard the results of IHA according to sex, the largest number of cases were positive in male group (19/26) cases (73%).

Analysis for *E. granulosus* anti-

bodies of patients' sera infected with different parasites; cross reactivity was found in one patient with *Fasciola* (1/6), and in another one with *S. mansoni* (1/6).

In this study, sensitivity, specificity, J-index, accuracy, Kappa, PPV, NPV were 86.67%, 95.0%, 81.67%, 91.43%, 0.824, 92.86%, 90.48% respectively.

IHA was previously used in diagnosis of hydatidosis (El Marsfy and Morsy, 1975; Antoniou *et al.*, 1995, 2002; Ramzy *et al.*, 1999; Liance *et al.*, 2000; Doiz *et al.*, 2002; Al-Sherbiny *et al.*, 2004; Magnaval *et al.*, 2004; Garin *et al.*, 2005; Gonlugur *et al.*, 2005; Mazyad *et al.*, 2007).



But, to the present authors' knowledge this test was once extensively evaluated in a clinical setting with a sensitivity of 87% and specificity of 98% (Force *et al.*, 1992), with the cut-off value recommended by the manufacturer. This agreed to some extent with the present results. The false-negative in echinococcosis was reported in 10%

to 20% of patients (Craig *et al.*, 2003; Zhang *et al.*, 2003).

Moreover, Morsy *et al.* (1983) reported cross reaction between the leishmaniasis IHA in dogs experimentally infected with *Toxoplasma gondii* and *E. granulosus*. Poretti *et al.* (1999) reported echinococcosis-IHA cross-reactions in the patients with malignancy (up to 19%) and

liver cirrhosis (25%).

Van-Doorn *et al.* (2007) stated that since crude extracts were used, cross-reactivity would be also expected in sera from the patients infected with other *Echinococcus* species.

In the present study, *E. granulosus* specific IgG was determined by ELISA, using the hydatid fluid antigen (cut-off OD value=0.394) only 1/30 patient gave negative result and 29/30 had positive reactions. Besides, 1/30 patient with *Fasciola* gave the positive cross reaction with other parasites. None of the controls gave positive reaction.

The sensitivity, specificity, J-index, accuracy, Kappa, PPV, NPV were 96.7%, 97.5%, 94.17%, 97.14%, 0.942, 96.67%, 97.5% respectively. Sensitivity from 81% to 96% and specificity between 84% & 100% for ELISA-IgG, attributing false positive to cross-reactions (Force *et al.*, 1992; Contreras *et al.*, 1994).

In the present study, ELISA sensitivity was comparable to or slight high than that reported earlier (Al-Yaman *et al.*, 1988; Craig, 1993; Moosa and Abdel-Hafez, 1994; Kaur *et al.*, 1999; Ramzy *et al.*, 1999; Zarzosa *et al.*, 1999; Sbihi *et al.*, 2001). In contrast, Hira *et al.* (1990) found a sensitivity of 98.1%. This variability in sensitivity might reflect the time lapse between surgical operation and sera collection. But, Manterola *et al.* (2005) reported sensitivity and specificity

for ELISA-IgG of lower values (83% & 87%, respectively).

The present authors stated that the differences could be explained partly by the characteristics of hydatid cysts as well as by the number of cystic lesions coexisting, since sensitivity increased in the presence of two or more cysts vs. solitary cyst (90% vs. 77%); cyst type, as in multivesicular cysts test was more sensitive than in univesicular ones (88% vs. 80%) and lesion size, as in cysts >15 cm sensitivity increased (85% vs. 80%).

In the present study, comparison of ROC curves of ELISA and IHA showed that ELISA-results were more sensitive than IHA as area under the ROC curve in ELISA was 0.992 while that of IHA was 0.974.

Nasrieh and Abdel-Hafez (2004) also, reported that the IHA was less sensitive than ELISA. So, ELISA proved far more superior to IHA as a serodiagnostic tests. Moreover, Kaur *et al.* (1999) and Ortona *et al.* (2000) reported that the ELISA sensitivity was significantly higher than that of IHA and that ELISA detected all IHA negative cases.

Conclusion

No doubt hydatidosis is a health and veterinary problem, not only in Egypt, but also worldwide. ELISA proved to be simple more sensitive and specificity as well as being not expensive.

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