

Indirect Haemagglutination as An Immunodiagnostic Technique For Cystic Echinococcosis In Iraqi Patients

Ali Ibrahim Ali Al-Ezzy *

Department of Pathology, College of Veterinary Medicine –Diyala University

Walaa Najm Abood

Department of Medical Microbiology, College of Medicine –Diyala University

Abstract:

Background: The aim is to determine the effectiveness of indirect Haemagglutination (IHA) as diagnostic techniques for Cystic Echinococcosis in Iraqi and possible correlation of age and gender in disease occurrence.

Patients and Methods: Patients presented with clinical manifestation of hydatidosis (202) were selectively enrolled in this study. IHA technique using sensitized sheep red blood cells coated with an *Echinococcus granulosus* antigen was applied.

Results:

One hundred and one patients (50%) were males, with the mean age (33.50 ± 7.8) years. The rest were females with mean age (29.78 ± 9.6) years. IHA positive cases were (19.3%) and the reminders (80.7%) were negative. IHA positive females (22.8%) compared with (15.8%) in males without statistical significant difference (P Value= 0.212) nor correlation between gender and IHA results (P Value= 0.214). The majority of patients belongs to (24-30) years, (31-36) years and (18-23) years age groups. Among males, higher number of positive cases at (31-36) years followed by (24-30) years and (49-54) years. Among females, higher number of positive cases at (24-30) years followed by (37-42) years and (18-23) years. Statistical significant difference (P Value= 0.001) reported between genders according to age group distribution without significant correlation between age group according to gender and IHA results (P Value= 0.268).

Conclusion: the IHA consider rapid, reliable and adequate technique that make it possible to process a large number of samples simultaneously and that do not require highly trained technical personnel. Females appear to be more susceptible to CE than male especially in younger age groups.

Key words: Indirect Haemagglutination, Hydatidosis, age, gender, Iraq

Introduction:

Cystic Echinococcosis a zoonotic disease that occurs throughout the world and causes economic losses and public health problems in many countries. Domestic intermediate hosts (sheep, goats, and cattle) are major reservoirs for the disease in humans. Infection of humans occurs during the natural transmission of the parasite between the definitive hosts and domestic livestock intermediate host [1].

Diagnosis of Cystic Echinococcosis is based currently on identification of the parasite's structures by imaging techniques, including ultrasound, computerized tomography and magnetic resonance imaging [2]. However, imaging techniques are relatively complex, do not always offer a good prospect for early diagnosis, and produce data that are sometimes difficult to interpret, being often confused with those from abscesses and neoplasm. In addition, imaging technology is not always available in developing countries with inadequately equipped

medical facilities [1]. Clinical symptoms do not appear until the larva of the parasite has reaches a certain size, which normally requires many years after the primary infection. Therefore, the clinical diagnosis is based only on assumption, and needs specific tests, For these reasons, serological methods are important not only for confirmation of CE case, but also to differentiate hydatidosis from other cystic lesions and tumors [1], also for epidemiological studies in endemic areas. Serological techniques are also very useful for the follow-up of patients after surgical or pharmacological treatment [1]. Serological tests for diagnosing hydatid infections in people living in areas where the disease is endemic are useful because of the low cost and ease of performance. Meanwhile, radiological techniques are often too expensive or are not available in many areas where hydatidosis is highly endemic [3]. The presence of raised specific antibody titers in patients with cystic hydatid disease has been assayed by various techniques, such as indirect haemagglutination(IHA) or latex agglutination, immunoelectrophoresis, complement fixation, immunoenzymatic, and indirect fluorescent antibody tests [4]. In many countries including Iraq hydatidosis is important both to human and animal health, because of the morbidity and occasionally mortality cases by the infection of humans with this disease.

The aim of this study is to determine the effectiveness of indirect haemagglutination as immunodiagnostic technique for hydatid cyst in Iraqi patients and possible correlation of age and gender in disease occurrence.

Patients, Materials and Methods:

Selection of patients:

Two hundred and two patients presented with clinical manifestation of hydatidosis attended to private and public outpatient's clinics in Baghdad during a period from June 2013 to May 2015 selectively enrolled in this study. This study was conducted according to the principles of Helsinki declaration. Dully-filled consent form obtained from all patients participating in the study. Approval of ethical review Committee of College of medicine – Diyala University-Iraq taken prior to initiation of the work.

Methods:

Five milliliters venous blood taken aseptically from two hundred and fifty one patients presented with clinical manifestation of hydatidosis. The samples centrifuged at $2000 \times g$ at room temperature for five min to separate sera. Separated sera were stored at -20 C° until used for indirect heamagglutination test (IHA) techniques.

Indirect heamagglutination test (IHA) techniques from Fumouze Diagnostics-France (www. Fumouze Diagnostics.com) [5]

Principle of the test based on sensitized sheep red blood cells coated with an Echinococcus granulosus antigen. Serum antibodies against Echinococcus granulosus are revealed by an agglutination of sensitized sheep red blood cells. A reddish brown film can be observed in the well. In the absence of specific antibodies, these red blood cells deposit forming a ring in the well bottom. The unsensitized red blood cells ensuring the reaction specificity and allow the elimination of interferences due to the reaction is performed in U-microplate. The test procedure is easy and rapid the results are obtained in two hours.

Statistical analysis:

Data were analyzed with by SPSS for window TM version 17 and Microsoft Excel for windows 2010 . Pearson’s chi square and Pearson’s Correlation coefficient used for correlation between the variables of two test .The level of significant was 0.05(two tail), and the significant of correlations include also 0.01 (two tail).

Results:

Table (1) shown the demography of (202) patients presented with clinical manifestation of hydatidosis .The mean age was (31.64±8.98) years, minimum age was (18) years and maximum age was (70) years. One hundred and one patients (50%) were males, with the mean age (33.50±7. 8) years. The rest of patients were females with mean age (29.78±9. 6) years, minimum age was (18) years and maximum age was (70) years. As shown in table (2), 39(19.3%) of cases were positive and the reminder 163(80.7%) were negative .Number of positive cases was higher among females (22.8%) compared with (15.8%) in males without any statistical significant difference (P Value= 0.212) nor correlation between gender and IHA results (P Value= 0.214) as shown in table(3).

As shown in table (4); The majority of patients belongs to (24-30) years, (31-36) years and (18-23) years age groups .Higher number of positive cases among males at (31-36) years followed by (24-30) years and (49-54) years. Among females, higher number of positive cases at (24-30) years followed by (37-42) years and (18-23) years. There was a statistical significant difference (P Value= 0.002) between males age groups distribution without significant correlation between males age groups and IHA results (P Value= 0.302). There was a statistical significant difference (P Value= 0.001) between females according to age group distribution without significant correlation between females age groups and IHA results (P Value= 0.340).There was a statistical significant difference (P Value= 0.001) between both genders according to age group distribution without significant correlation between age group according to gender and IHA results (P Value= 0.268) as shown in table(4).

Table (1) Demography of patients with clinical manifestations of hydatidosis

Parameter	Parameter	
Age (years)	Minimum	18
	Maximum	70
	Mean ±SD	31.64 ±8.98
Gender	male	101(50%)
	female	101(50%)
Age according to gender	Male	Female
Minimum (years)	20	18
Maximum(years)	56	70
Mean ±SD	33.50±7.86	29.78±9.6

Table (2): Results of IHA test in patients presented with cystic hydatidosis

IHA test	No.(%)
Positive cases	39(19.3%)
Negative cases	163(80.7%)
Total	202(100%)

Table (3): IHA test according to gender of patients presented with cystic hydatidosis

Gender	IHA test		X2		Pearson's correlation	
	Positive No.(%)	Negative No.(%)	value	P Value	value	P Value
Male	16(15.8%)	85(84.2%)	1.557	0.212	-.088	0.214
Female	23(22.8%)	78(77.2%)				
Total	39(19.3%)	163(80.7%)				

Table (4): IHA test according to age group of patients presented with cystic hydatidosis

Age group (years)	Total No. (%)	IHA test						X2		Pearson's correlation	
		Male			Female			Value	P value	Value	P value
		Total male	positive IHA	negative IHA	Total Female	positive IHA	negative IHA				
18-23	42 (21.56%)	7(3.47%)	2 (0.99%)	5 (2.48%)	35 (17.33%)	6 (2.97%)	29 (14.36%)	60.550	0.001	-0.078	0.268
24-30	55 (27.22%)	32 (15.84%)	4 (1.98%)	28 (13.86%)	24 (11.88%)	9 (3.96%)	15 (7.43%)				
31-36	48 (23.76%)	34 (16.83%)	6 (2.97%)	28 (13.86%)	15 (7.43%)	0 (0%)	15 (7.43%)				
37-42	34 (16.83%)	14 (6.93%)	0(0%)	14 (6.93%)	19 (9.41%)	7 (3.47%)	12 (5.94%)				
43-48	13 (6.43%)	8 (3.96%)	0 (0%)	8 (3.96%)	5 (2.48%)	0 (0%)	5 (2.48%)				
49-54	7 (3.43%)	4 (1.98%)	4 (1.98%)	0 (0%)	2 (0.99%)	0 (0%)	2 (0.99%)				
55-61	2 (0.98%)	2 (0.99%)	0 (0%)	2 (0.99%)	0 (0%)	0 (0%)	0 (0%)				
62-67	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)				
68-73	1 (0.49%)	0(0%)	0(0%)	0(0%)	1(0.5%)	1(0.5%)	0 (0%)				
Total	202 (100%)	101 (50%)	16 (7.92%)	85 (42.08%)	101 (50%)	23 (11.39%)	78 (38.61%)				
X2 value		47.870			55.296						
P value		0.002			.001						
Pearson's correlation value		-0.104			-.096						
P value		0.302			0.340						

Discussion:

E. granulosus is known to be endemic in several areas of the world and cystic echinococcosis is often considered as a disease of developing countries and of populations with low socio-economic status. Hydatid cyst disease is an extensive epidemiological problem in developing countries like Iraq, particularly in the cattle and sheep raising areas. In the present study 202 patients presented with clinical manifestation of hydatidosis were enrolled. The minimum age was (18) years while the maximum age of patients was (70) years with mean age (30.82 ± 0.52) years. This result come in accordance with recent Iraqi study [6], in Ethiopian study the age quite near of that in Iraq (39.33 ± 2.28) years, study [7] and disagree with others reporting incidence in age $(61.3 + 17.1)$ years [8]. The outstanding result in current study was registration of CE among younger age groups (24-29), (30-36) and (37-42) years compared with reports from northern Iraq [1], Latin America [8] and china [9] in which CE more frequent among older ages (60-69) and (70-79) years.

The disease occurs in males and females, the sex ratio being different in the different endemic areas, depending on the characteristics of the parasite cycle in this area, and of particular behaviors in the communities. In the present study male patients represent (50%), females (50%) without significant differences (p value=0.212) between IHA positive and negative cases. This result disagree with that reported in Qatar [10] and Egypt [11]. However in china and Argentina a higher prevalence was reported in females [8, 12]. Housewives, especially in rural areas, where the most infected cases can be found, have the highest chance of contact with the sources of infection. Contact with contaminated vegetables, cleaning the house containing the dog faeces, desire to eat soil (Geophagy) as longing in pregnant embrace the etiological issues, highly infection rate in females is in agreement with most of other studies which have shown a high frequency in females [6, 13, 14]

In this study, the overall prevalence of echinococcosis in humans was (19.3%) . These results are higher than findings of some workers who reported the prevalence rate ranged from 2.3 to 8.5 percent, and it's lower than findings of [13-16]. The results of IHA indicate higher incidence in females (22.8%) than males (15.8%).

In this study, younger age groups appear to be more frequently infected among males in which, IHA for CE was positive in (2.97%) among males at the age group (31-36) years; less frequently (1.98%) among (24-30) years and (49-54) years age group. There was a statistical significant difference (P Value= 0.002) between males according to age group distribution without significant correlation between males age group and IHA results (P Value= 0.302). At the same time, younger age groups appear to be more frequently infected among females in which, IHA for CE was detected in (3.96%) at age group (24-30) years, (3.47%) in (37-42) years and less frequently (2.97%) among (18-23) years and (49-54) years age group which come in line with other studies in Yemen [13, 17]. There was a statistical significant difference (P Value= 0.001) between females according to age group distribution without significant correlation between age group according to female gender and IHA results (P Value= 0.340). There was a statistical significant difference (P Value= 0.001) between both genders according to age group distribution without significant correlation between age group according to gender and IHA results (P Value= 0.268). The highest risk group in the country is specifically in Arab Gulf region -in general- which are women and children. Traditionally, rural women especially of a younger age groups; still bear the biggest burden of tending ani-

mals – whether breeding, milking, or wool -shearing- and usually in such environment a domestic or stray dogs are never far away. The added chore of women preparing and cooking contaminated food and vegetables with little clean water at hand increases considerably the risk of infection. In many parts of middle east during springtime, it is common practice to gether berries and various wild plants which are eaten unwashed [6].

Conclusion:

The IHA consider rapid, reliable and adequate technique that make it possible to process a large number of samples simultaneously and that do not require highly trained technical personnel. Females appear to be more susceptible to CE than male especially in younger age groups.

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