



Seroepidemiological Study of Hydatid Cyst Using AgB by ELISA in Patients Admitted to Central Laboratory of Baqiyatallah Hospital

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Abstract

Background: One of the most important parasitic diseases in human is cystic echinococcosis (CE) which is caused by the larval stage of *Echinococcus granulosus*. The disease is a health problem in the Middle East countries such as Iran.

Objective: The aim of this study was seroepidemiological evaluation of human CE using antigen B by ELISA technique in patients referred to Baqiyatallah Hospital, Tehran, Iran.

Materials and Methods: A total of 909 serum samples were randomly prepared in the central laboratory. Antigen B was extracted from sheep hydatid cyst fluid (HCF). All serum samples were evaluated by indirect ELISA and the suspected cases were rechecked. Demographic characteristics were collected by a questionnaire and cut-off was calculated as $X \pm 2SD$.

Results: In the present study, 0.8% of the collected samples were seropositive by ELISA technique. Of the 909 participants, 498 (with 1% positive response) and 411 (with 0.49% positive response) were female and male, respectively. The majority of the cases were the employee and retired military personnel (with 1.05% positive response). The most frequent age ranges were 50-59 and 70-79 with 28.57% positive response in each and in participants with academic education (85.72%). There was no statistically significant association between hydatid cyst and variables evaluated in this study.

Conclusion: Our results showed a low seroprevalence of human CE. It may be related to the lifestyle of people following the improvement in public health specially in urban communities.

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Background

Cystic echinococcosis (CE) is an important zoonotic disease caused by metacestode or larval stage of *Echinococcus granulosus* in human and different ruminants. The adult stage of the parasite is observed in canids throughout the world, particularly in countries with a higher number of susceptible animals. The infection has considerable importance in medical, veterinary and economic aspects.¹⁻⁵

The incidence of infection is high in humans in countries with pastures in which there is intimate contact with dogs such as Australia, New Zealand, southern South America, North Africa, the Mediterranean littoral, Eastern Europe, and the Far East. Iran is located in

an endemic/hyperendemic area in the Middle East in which various species of animal become infected by *E. granulosus*.^{4,5} Moreover, human CE is also reported in several studies in different cities of Iran.^{4,6-10}

Usually, the disease remains asymptomatic for a long time and the diagnosis is difficult at the early stage of the disease. The assessment of CE has been carried out by pathognomonic features utilizing radiologic techniques including ultrasonography, computed tomography (CT) and magnetic resonance imaging (MRI). The seroimmunological methods along with imaging finding are strongly recommended by the World Health Organization (WHO) for evaluation of the disease.^{5,11} ELISA has been reported to be a relatively reliable test

for diagnosis of the disease, especially in epidemiological studies, with considerable sensitivity and specificity. Until now, different antigens have been used for serodiagnosis of CE. Antigen B (AgB) is the main semipurified lipoprotein originated from hydatid cyst fluid (HCF) which has been used in many studies.^{7-10,12}

Objectives

Since the epidemiological studies are the basis for monitoring of prevention protocols, this study was designed to assess the ELISA method using AgB for immunodiagnosis of human hydatid cyst in the patients admitted to Baqiyatallah Hospital, Tehran, Iran.

Materials and Methods

Sample Collection

A total of 909 sera samples were randomly collected from patients who referred to the central laboratory (March to October 2016). Five surgically, pathologically and serologically confirmed CE samples and 30 sera samples from healthy subjects were utilized as positive and negative control respectively.

Preparation of Hydatid Cyst Fluid

HCF was obtained from the infected sheep in industrial abattoirs in Semnan and Qom. To remove large particles, HCF was centrifuged at 3000 × g for 15 minutes at 4°C and stored at - 20°C until use.

Preparation of Antigen B

Antigen B was prepared from HCF as described by Oriol.¹³ Briefly, 100 mL of HCF was dialyzed overnight against 5 mM of acetate buffer (pH 5) at 4°C. Samples were centrifuged (50 000 g for 30 minutes), supernatant was removed and the pellet was dissolved in 0.2 M phosphate buffer (pH 8). Saturated ammonium sulfate was used to remove the globulins from the sample.

Finally, the sample was boiled in a water bath for 15 minutes and centrifuged at 50 000 g for 60 minutes to separate heat-stable antigen B (supernatant) from other components. Concentration was determined by Bradford protein assay.

Enzyme-Linked Immunosorbent Assay

Enzyme-linked immunosorbent assay (ELISA) was carried out in flat-bottom 96-well microplates. The plate wells were coated with 5 µg/mL of AgB (100 µL/well) in

coating buffer (0.05 M carbonate-bicarbonate buffer, pH 9.6) and incubated at 4°C overnight. Excess antigen was removed by washing the plate three times in phosphate buffered saline-Tween 20 (PBST, pH 7.4 containing 0.05% Tween 20). Blocking was done with 5% skimmed milk in PBST for 1:30 hours. The wells were washed and 100 µL of serum sample (1/100 dilution in PBST) was incubated for 2 hours. The plates were washed 5 times and 100 µL anti-human IgG (Sigma, USA) (1/4000 dilution in PBST) was added to the plates and incubated for 1 hour at room temperature. After washing as before, the plates were incubated with chromogen/substrate (100 µL/well of OPD, 0.025% H₂O₂ in 0.1 M citrate buffer, pH 5). The absorbance was read at 492 nm after 30 minutes using automatic microplate reader.

Statistical Analysis

All data were analyzed using SPSS software version 21.0 (Chicago, IL, USA). Student's *t* test was developed and a *P* value less than 0.05 was considered significant. The cut-off was calculated as $X \pm 2SD$.

Results

In the present study, 0.8% of the collected samples were seropositive by ELISA test. Of the 909 participants, 498 (with 1% positive response) and 411 (with 0.49% positive response) were female and male, respectively (Table 1).

The majority of the cases in the present study were the employed and retired military personnel (287 persons with 1.05% positive response) followed by the housewife group (375 persons with 0.53% positive response) (Table 2).

The lowest and highest ages of the cases were 1 and 86 years old with an average age of 46.07 years. The most frequent age ranges were 50-59 and 70-79 with 28.57% positive responses in each (Table 2).

The educational level varied from illiteracy to doctoral degree. The most frequent positive responses were in participants with academic education (85.72%) (Table 2). There was no statistically significant association between hydatid cyst seropositivity and variables evaluated in this study, probably because of a low prevalence.

Discussion

CE is one of the most important zoonotic diseases and it is endemic in several countries worldwide.⁴⁻⁶ Moreover, it is prevalent in final and intermediate hosts in different part

Table 1. The Seroprevalence of Human Hydatidosis in Baqiyatallah Hospital Based on Sex (March-October 2016)

Sex Distribution	Positive Responses	Negative Responses	Total
	No. (%)	No. (%)	No. (%)
Female	5 (71.43)	493 (54.66)	498 (54.79)
Male	2 (28.57)	409 (45.34)	411 (45.21)
Total	7 (100)	902 (100)	909 (100)

P > 0.05.

Table 2. The Seroprevalence of Human Hydatidosis in Baqiyatallah Hospital Based on Educational Level, Age and Occupation (March to October 2016)

Variables	Positive responses No (%)	Negative responses No (%)	Total No (%)
Educational level			
Illiterate	1 (14.28)	63 (6.99)	64 (7.04)
Elementary	0 (0)	69 (7.65)	69 (7.59)
Guidance school	0 (0)	104 (11.52)	104 (11.44)
Diploma	3 (42.85)	286 (31.71)	289 (31.80)
Associate degree	0 (0)	81 (8.98)	81 (8.9)
Bachelor's degree	2 (28.56)	252 (27.94)	254 (27.94)
Master's degree	1(14.28)	35 (3.88)	36 (3.96)
PhD	0 (0)	12 (1.33)	12 (1.32)
Age			
<10	0(0)	6 (0.67)	6 (0.66)
10-19	1(14.28)	46 (5.1)	47 (5.17)
20-29	0(0)	141 (15.63)	141 (15.51)
30-39	0(0)	135 (14.96)	135 (14.85)
40-49	1(14.28)	134 (14.85)	135 (14.85)
50-59	2 (28.57)	253 (28.04)	255 (28.05)
60-69	1(14.28)	119 (13.19)	120 (13.2)
70-79	2 (28.57)	53 (5.87)	55 (6.05)
80-89	0(0)	15 (1.66)	15 (1.65)
Occupation			
Housewife	2 (28.57)	373(41.35)	375 (41.25)
Student	1 (14.29)	22 (2.44)	23 (2.53)
Employee	1 (14.29)	23 (2.55)	24 (2.64)
Military personnel	3 (42.85)	284 (31.48)	287 (31.57)
Others	0 (0)	200 (22.18)	200 (22.01)

$P > 0.05$.

of Iran.^{7-10,14-18}

Our results showed that 0.8% of participants were seropositive for hydatid cyst disease. The assessment of many studies demonstrated that the seroprevalence of CE varied between 0.22% in Tehran¹⁸ and 15.4% in Khoramabad.⁹ Although this indicator in our study is much less than many other studies in the country, it seems to be logical in Tehran as a metropolis. In this regard, our results are a little higher than the findings obtained from Shemiranat region by Farrokhzad et al (0.22%) by IFA method¹⁸ and are slightly less than those obtained in Transfusion Center by Akhlaghi et al (1.6%).¹⁹

Many investigations were shown that hydatid cyst usually occurs in adulthood/ middle age and was generally increased with the increase of the age.^{14,20,21} In the present study, although the most frequent positive response was observed in 40-79 (85.72%) age group, there is not any significant differences between age and seroprevalence of the disease.

The prevalence of the disease was significantly lower in males than in females in different investigations.^{6,14} In our study, 1% of the 498 females and 0.49% of 411 males showed positive response using AgB by ELISA test, but the relationship between gender and prevalence was not significant.

The housewives encompass the highest rate of infection

in different investigations. This group of women, especially in villages, had more contact with the sources of infection, because of some responsibilities, like cleaning vegetables. The desire to eat soil (geophagia or pica) in pregnant women also increases the possibility of contamination.⁶ The majority of the cases in the present study were the employed and retired military personnel (287 persons, with 1.05% positive response) followed by the housewife group (375 persons, with 0.53% positive response). As there is not much information about the outbreak of the disease in the armed forces, the comparison of this job is not possible.

Although some studies reveal that CE is more prevalent in low-educated people,²²⁻²⁴ in our investigation, higher seropositive rate is observed in diploma and bachelor's degree. It is considered that seroprevalence is more related to knowledge and beliefs about the disease and transmission models rather than educational status alone.

In conclusion, the low incidence of hydatidosis in the present study in comparison with some other previous reports could be related to the changes in lifestyles following the improvement in public health in urban communities.

Authors' Contributions

Study concept and design: TM; Acquisition of data and serum sampling: ME; Hydatid cyst preparation: SF; HCF and Ag preparation: TM, ME, RY; ELISA technique: ME, KA, TM; Analysis and interpretation of data: MS, ME, TM; Drafting the manuscript: TM, ME, RY.

Ethical Approval

Approval of the study protocol was received from the Ethical Committee of Baqiyatallah University of Medical Sciences (No: IR. BMSU.IEC.1396.536).

Conflict of Interest Disclosures

The authors declare that they have no conflict of interests.

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