



Seroepidemiology of Human Hydatidosis Using AgB-ELISA Test in Arak, Central Iran

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Abstract

Background: On the continuation of sorting the puzzle of the situation of hydatid disease in Iran and considering that so far no survey was conducted in this context in Arak City, Markazi Province central Iran, the present study was conducted to determine the seroprevalence of human hydatidosis using AgB-ELISA test.

Methods: Totally 578 serum samples randomly were collected from patients referred to hospitals and different health centers in the city and 3 nearby villages of Arak. All sera were examined by ELISA tests using AgB. Before sampling, a questionnaire was filled out for each case. Data were analyzed using Chi-square test and multivariate logistic regression for risk factors analysis. $P < 0.05$ was considered significant.

Results: Cut-off value was calculated 0.32. Twenty cases (3.46%) were seropositive for hydatidosis in the region. This rate for females was 3.99% and for males 2.26%, respectively. There was no significant difference as regards all factors studied except location ($P < 0.001$). As for job, farmers and ranchmen had the highest rate of infection as 6.67%. The seropositivity rate infection was 4.8% in illiterate people, which showed the highest rate. As regards residency, rural life showed significant difference with urban life (1.5% vs. 7%). Age group of 40-49 yr old (6.25%) had the highest rate of positivity.

Conclusion: The rate of prevalence in this region shows more or less the same range with other cities of Iran. Obtained result might assist the policy makers to take sanitary measures to control the disease.

Keywords: Seroepidemiology, Human hydatidosis, ELISA, Iran

Introduction

Cystic echinococcosis (CE) or hydatid disease is a silent zoonotic infection that has importance major public health not only in humans but in veterinary field. The disease is established through ingestion of the parasite's egg via various vehicles such as soil, vegetables, contact with dog, air, etc. The agent called *Echinococcus granulosus* is lodged in the intestine of final host as canids (1).

Two various forms of cysts, entitled, unilocular cyst and multilocular cyst (produced by *E. granulosus* and *E. multilocularis*, respectively) are among the

most important human diseases in terms of imposed DALYs and economical damages (2).

This multi-host parasite has a worldwide dispersion and is one of the most important zoonotic diseases prevalent in all over the world and is regarded endemic in the entire Mediterranean zone including all countries from the Middle East (1,3). Based on literature, Iran is one of the endemic areas with human infection rate of $>1\%$ of total population of country (4). Hydatidosis and dogs infection have been reported approximately from

entire Iran but drier areas in the south show less infectivity than northern areas. According to these studies the rate of prevalence has been reported from 1.6% to 49% in different provinces (5-9).

The diagnosis of hydatidosis is based on a category of scanning and immunological methods because there is no output of the parasite in stool or urine of the patients (10-12). Many previous studies have based their seroprevalence surveys on methods such as ELISA but the problem is that antibody remains for years in patients' sera and impossible easily to differentiate the acute or chronic phase of the disease or better to say to follow up the patient after treatment or surgery. (13).

The optimal serological test for the diagnosis of hydatidosis is enzyme-linked immune sorbent assay (ELISA). Specifically among parasite's antigens, AgB resistant to 100 °C temperature is the most prominent test which has been used by many researchers so far with authentic outcomes (14-18), although some challenges have been pro-

posed, mostly the weak point of not capable to follow up the patients (19, 20).

Considering that so far there are no authentic data on the prevalence of hydatid disease in Arak Province, central Iran, the present study was undertaken using AgB-ELISA to assist on setting another piece of above-mentioned puzzle.

Materials and Methods

Study Area

Arak City located in central Iran in Markazi Province, and surrounded by mountains in the west, east and south with *and latitude*, 34° 19' 59 N and longitude of 49° 49' 59 E. It is the center of province and the population is 76923300 according to the last census in 2006 that at least one fifth of them live in the urban areas (<http://www.horologeparlante.com/time-ost%C4%81n-e-markaz%C4%AB-iran-TIMEus124763r.html>). The city consists of 4 urban and 9 rural regions (Fig. 1).



Fig. 1: Map of Iran and Arak as the studies area

Samples

Overall, 578 Serum samples were randomly collected according to consultant with statistician from cases including 177 males and 401 females referred to clinical and health centers, as well as private and governmental laboratories in Arak. Although based on the statistician consulting the volume of sample was determined as 458, but for more caution, overall 578 people were enrolled in the study. To select the cases, from each geographical region of the city and nearby villages, sampling was conducted using different clusters and simple sampling. In each cluster from the villages of Ebahimabad, Amanabad, Davoudabad and one region of the city including Arak (Arak, Senejan, and Karahrud) sampling was conducted. Sera were stored in refrigerator at -20°C , and then were sent to Dept. of Medical Parasitology, School of Public Health, Tehran University of Medical Sciences, Iran for examination with ELISA.

A questioner was filled out for each individual for getting information including various factors such as age, sex, job, locality, literacy and so on that effect on the disease. An informed consent was taken from each participant and from their parents if they were child. The study was approved by Ethical Committee of Tehran University of Medical Sciences.

Antigen

At first, hydatid cyst fluid (HCF Ag) was aspirated from hydatid cysts obtained from livers and lungs of sheep slaughtered at the local abattoirs of Tehran. Antigen B was purified and extracted as described earlier (16).

ELISA test

ELISA test was performed in 96 well micro plates (Nunc, Denmark) as previously described (16), with some modifications. Micro plate wells were coated overnight at 4°C with $100\ \mu\text{l}$ AgB ($10\ \mu\text{g}/\text{ml}$) in $0.05\ \text{M}$ bicarbonate buffer, pH 9.6. Wells were washed 3 times in PBS plus 0.05% Tween 20 (PBS-T) and blocked with PBS-T containing 1% BSA for 30 min at 37°C . Sera were added at 1:500 dilutions in PBST, incubated at

37°C for 1 h then washed as before. Antihuman IgG -HRP (Sigma Chemical Co., Poole, Dorset, United Kingdom) conjugates were added at 1:10000 dilutions in PBS-T and the micro plate incubated and washed as before. This was then developed by OPD substrate ($5\ \text{mg}$ 1, 2-phenylenediamine, $12.5\ \text{ml}$ of $0.2\ \text{M}$ citrate phosphate buffer pH 5, $10\ \mu\text{l}$ 30% H_2O_2). The absorbance was read at $492\ \text{nm}$ after 10 min using an automatic micro plate reader (State Fax® 2100, Awareness, USA). Altogether 30 samples of sera from healthy volunteers had been collected during the previous studies were examined to set the cut-off. Cut-off was calculated as mean + 3 SD.

Data analysis

All data were analyzed using SPSS software ver. 16 (Chicago, USA). *P* value less than 0.05 was considered as significant.

Results

The result of seroprevalence study of human hydatidosis was detected as 3.46% (twenty cases) by ELISA test in this area (Fig. 2). Cut-off was calculated as 0.33.

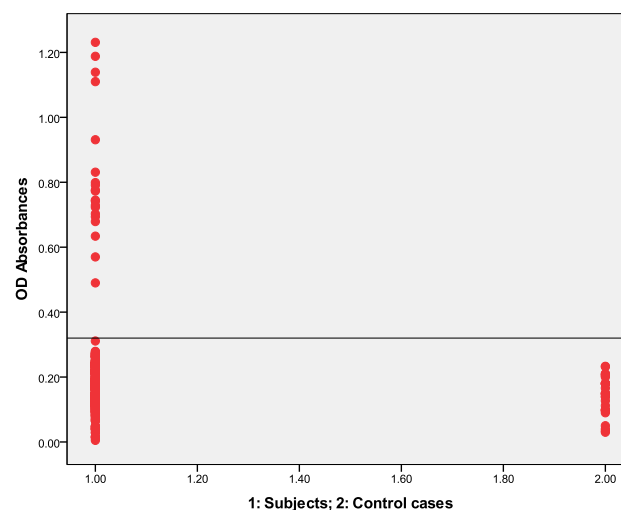


Fig. 2 : Analysis of sera from subjects and normal controls from Arak City, Markazi Province, central Iran by IgG-ELISA using Antigen B. Serum samples obtained from subjects (578, Lanes 1), and normal controls (30, Lanes 2)

Rural areas prevalence was significantly higher (6.98%) than the urban area (1.46 %) ($P < 0.001$). There was no significant association between CE seropositivity and age group, occupation, sex, literacy, contact with dog and unwashed vegetables. The prevalence among females (4.15%) was higher than males (2.31%). As to the age group the highest rate was detected in 40-49 yr group (6.25%). Other age group's infectivity is obvious in Table (1).

Table 1: Distribution of positive cases of hydatidosis using ELISA according to age group (yr) in Arak, Markazi Province, Iran

Age group (yr)	Sample taken (n)	Positivity (n)	Sero-Prevalence (%)
9>	9	0	0
10-19	50	0	0
20-29	95	3	3.15
30-39	121	4	3.30
40-49	96	6	6.25
50-59	85	4	4.70
60-69	73	2	2.74
70<	46	1	2.17
Total	20	578	3.46

As regards occupations, farmers and ranchmen had the highest rate of infection (6.67%). The rate of the disease was 4.96% in people having history of contacting with dog but 2.17% in people with negative history in this subject. The prevalence of infection based on literacy was 4.76%, 2.85 %, 2.23% as for illiterate, diploma and graduated people, respectively. People having history of eating unwashed vegetable showed 3.97% seropositivity. Singles and married cases showed 4.14% and 2.34% of the positivity with no significant difference. The prevalence of disease in people with and without history of geophagy was 9.01% and 3.47%, respectively.

Discussion

Total prevalence of human hydatidosis was 3.46% (Twenty cases) in this survey using ELISA. Incidence of human CE annually almost ranges from 1 to 200 per 100,000 residents in the world (21).

The annual incidence in Iran from various cities are as follows: Hamadan 1.33/100000, Kashan (Isfahan) 3/100000, Babol 1.18/100000 and entire of Iran 0.61/100000 (17, 18, 22, 23).

Previous studies based on different serological examination from Various parts of the country showed different amount of prevalence rate as: Zanjan 3% (24), nomads tribes from south of Iran 13.7% (25), Ilam 1.2% (26) , Kashan 2.04% (17), Sanandaj 7.3% (27), Golestan 2.34% (7), Meshkinshahr 1.79% (8) , and Qom 1.6% (9). The rate of infection in our study (3.46%) shows more or less a similar rate with other parts of Iran. The method of survey in these studies was almost the same, i.e. using ELISA. One of the most important key roles in spreading hydatidosis is contact with dogs. In a vast study done in the 13 provinces of Iran, the prevalence of hydatidosis in the sheepdogs was 27.17% (28). Overall, infection rate of hydatidosis in stray dogs ranged from 5 to 4% in different parts of Iran (5), western Iran as 20% (29) and Kurdistan Province 44% (27). Stray dogs are freely spread in different parts of Arak which is one key factor of increasing the risk of infecting with the disease.

In this study, the highest rate of infection was in age group of 40-49 yr old (6.45%) which is similar to the results of Baharsefat et al. (7). Previous studies have reported the 10-19 year old as the highest infected age group in Zanjan (24), 60-80 yr old in Hamadan (4), and 20-40 yr old in Kurdistan (30). In addition, age groups of 20-30, 60-90 and 30-60 yr old have been reported as the highest rate of infection in Kerman, Meshkinshahr and Qom, respectively (8, 9, 31). The problem is that the prepatent period is very long in this disease and most cases are diagnoses years after infection (4). So it is impossible to detect the true age group in the context of infection with hydatidosis.

Females were more infected than males (4.15% vs. 2.31%) like most of studies conducted in Iran (7-9). Some different studies in Iran showed a higher rate of infection to hydatid disease in males than females (8-9, 32-33). Women have the highest chance of contact with sources of infection such as dog, soil, vegetable etc. As to occupation, we noticed that farmers and ranchmen (6.67%), pos-

sessed the highest rate. This might be due to the culture of the area, where men are in more contact with risk factors than women might. In this study, illiterates showed the highest rate of infection (4.76%). The factor of residency demonstrated a significant difference in this study ($P < 0.001$) similar to earlier studies that showed significant difference between rural life with urban life (33-35).

This study is a part of wide plan on detection the epidemiology of hydatidosis in Iran, which can help us to have a comprehensive view about present situation, and future planning of disease.

Conclusion

The rate of prevalence in Arak shows more or less the same range with other cities of Iran. Results prove that hydatidosis already is one of the notable public health topics and despite the important data and the socioeconomic impact (36-39), echinococcosis remains a neglected disease and must be considered by authorities in public health domain.

Ethical considerations

Ethical issues (Including plagiarism, Informed Consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc) have been completely observed by the authors.

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