Iran J Public Health, Vol. 50, No.3, Mar 2021, pp.592-597

Original Article

Seroprevalence of Cystic Echinococcosis Using Recombinant Antigen B-ELISA in North Khorasan Province, Northeast of Iran

Reza SHAFIEI [1,2], Fatemeh TAGHASI [3], Seyed Ahmad HASHEMI [2], Yaser PANAHI [4], Nasir AREFKHAH [5], Mostafa OMIDIAN [6], Farzane ARIANFAR [7], Zohreh MOSTAFAVI-POUR [7], *Bahador SARKARI [8]

1. *Vector-Borne Diseases Research Center, North Khorasan University of Medical Sciences, Bojnurd, Iran*
2. *Department of Pathobiology and Laboratory Sciences, North Khorasan University of Medical Sciences, Bojnurd, Iran*
3. *Student Research Committee, North Khorasan University of Medical Sciences, Bojnurd, Iran*
4. *Laboratory Affairs, Health Assistances, North Khorasan University of Medical Sciences, Bojnurd, Iran*
5. *Cellular and Molecular Research Center, Yasuj University of Medical Sciences, Yasuj, Iran*
6. *Department of Parasitology and Mycology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran*
7. *Department of Biochemistry, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran*
8. *Basic Sciences in Infectious Diseases Research Center, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran*

***Corresponding Author:** Email: sarkarib@sums.ac.ir

(Received 14 Jan 2020; accepted 20 Mar 2020)

Abstract

Background: Cystic echinococcosis (CE) is one of the most important helminthic parasitic diseases in Iran. The current study aimed to assess the seroprevalence of CE in North Khorasan Province, Northeast of Iran in 2018.

Methods: The study was carried out in seven cities of North Khorasan Province. Venous blood samples were collected from 932 individuals referring to health centers of those seven cities. A questionnaire was used to obtain the data regarding the subject's gender, age, residence and risk factors linked to the hydatid cyst. Sera samples were evaluated for anti-hydatid cyst antibodies in an ELISA system, using a recombinant B8/1 antigen of *E. granulosus*.

Results: Of the 932 recruited subjects, 496 (53.2%) were male and 436 (46.8%) were female. The range of participants' age was between 11 to 83 yr old and the mean age of the subjects was 35.4 (±12.7) years. Anti-hydatid cyst antibodies were detected in the sera of 37 out of 932 subjects, corresponding to a seroprevalence rate of 3.96%. From these, 20 (54.05%) were male and 17 (45.95%) were female. There were no associations between seropositivity to hydatid cyst and age, the gender of the participants, residential areas and having contact with dogs (P>0.05).

Conclusion: CE is relatively prevalent throughout the North Khorasan Province in the Northeast of Iran. Rate of CE infection in this Province is somewhat similar to the rate of infection in other parts of the country.

Keywords: Seroprevalence; Cystic echinococcosis; Recombinant antigen B-ELISA; Iran

Introduction

Cystic echinococcosis (CE) is a zoonotic infection of humans and domestic animals caused by the larval stages of the dog tapeworm *Echinococcus granulosus*. The human becomes infected through

ingestion of foods contaminated with the *E. granulosus* egg defecated from infected dogs (1). CE is the most important chronic zoonotic helminthic infection with high prevalence in humans and livestock in some areas of the world with economic and public health impacts.

The greatest prevalence of CE in human and animal hosts is found in countries of the temperate zones, including several parts of Eurasia (the Mediterranean regions, southern and central parts of Russia, central Asia, and China), Australia, some parts of America (especially South America) and north and east Africa. The diseases are a serious health problem in the Mediterranean region, especially in countries in the Middle East including Turkey and Iran (1, 2). The seroprevalence rate of human CE in Iran varies from 1.6% up to more than 20% and the pooled seroprevalence of CE has been estimated at 6.0% (3-6).

Seroprevalence rates of CE in Iran have been reported to be 3.4% in Alborz Province in central Iran, 2.6% in Lorestan Province in the west, 1.79% in Meshkin-Shahr in the Northwest and 7.2% in the Yasuj area in the southwest of the country (5, 7-9). The number of surgeries due to CE in Iran has been reported to be 1300 cases (10).

Native antigen B has been used in most of the studies about the seroprevalence of CE. This antigen is appropriate in the diagnosis of hydatid cysts (11). In the present study, a recombinant antigen from one of the antigen B subunits has been used (12). This antigen has a slightly higher sensitivity and specificity than the native antigen B in the diagnosis of CE. Accordingly, this antigen was used in this study and a similar study regarding the seroprevalence of hydatid cyst in children in a rural area in Fars Province (13).

As there is no available data on the seroprevalence of CE in North Khorasan Province, northeast Iran, the present study aimed to assess the seroprevalence of CE in this area.

Materials and Methods

Study area and sampling

The study was carried out in 2018 in seven cities of North Khorasan Province, including Bojnurd, Shirvan, Maneh and Samalghan, Faruj, Raz and Jargaran, Garmeh and Jajarm (Fig. 1).

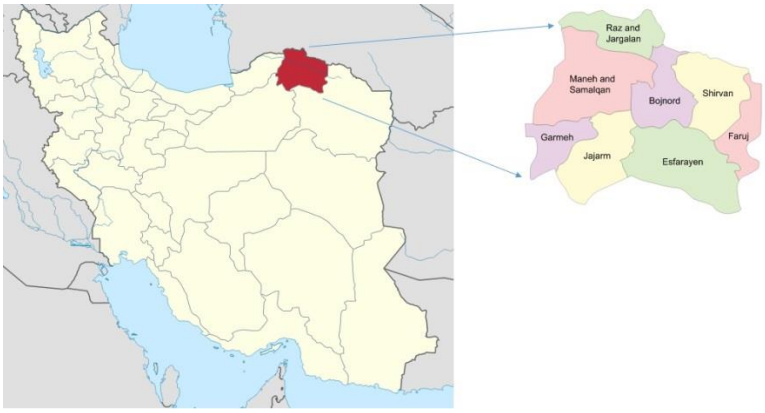

Fig. 1: Map of Iran showing North Khorasan Province and its counties

North Khorasan is of the 31 provinces of Iran located in northeastern of the country and Bojnurd is the capital of the province. North Khorasan covers an area of 28,434 km^2 and has a population of 850,000. The geographical coordinates of the province are 37°28'34"N and 57°19'54"E. North Khorasan holds diverse climate including the cities that enjoy moderate mountain climate and cities that face the desert. The population living in this area is living mainly on farming and animal husbandry.

Venous whole blood samples (about 5 mL) were collected from 932 individuals referring to health centers of the aforementioned cities. Sera were

separated from the blood samples and kept at -20 °C till use. The enrollment criteria were being the resident of the studied area and having no infectious diseases, while the exclusion criteria were having a fever, normally not feeling healthy, and unwilling to provide the blood.
Through sampling, a questionnaire was used to obtain the data regarding the subject's gender, age, residence and risk factors linked to the hydatid cyst such as having contact with the dog.

Detection of anti-hydatid cyst antibodies

Sera samples were evaluated for anti-hydatid cyst antibodies in an ELISA system, using a recombinant B8/1 antigen of *E. granulosus* (12). The ELISA was performed in Department of Parasitology and Mycology at Shiraz University of Medical Sciences, Shiraz, Iran. Briefly, the ELISA, flat-bottom 96-well microplates were coated (100 µL/well) with 5 µg/mL of the recombinant antigen in coating buffer. Unbound antigens were removed by washing buffer and blocking was performed, using 3% skimmed milk. Serum samples were added to the plates and the plates were washed after 2 hours. Horseradish peroxidase-conjugated anti-human antibody was added to the plates and were then washed after 1.5 hours. The substrate was added to the plate and the absorbance was measured positive sera (from pathologically confirmed hydatid cyst patients) along with sera from healthy controls were applied in each run of ELISA and the cutoff point was set at 2 standard deviations (SD) above the mean OD of the negative control samples.

Statistical analyses

Statistical analysis of the data was performed using SPSS (ver. 18, Chicago, IL, USA). The association between the seropositivity to hydatid cyst and the subjects' demographic features and also the risk factors for the infection were verified, utilizing the Chi-Square statistic.

Ethical approval

The study was approved by the Ethical Review Committee of North Khorasan University of Medical Sciences (ethical code: IR.NKUMS.Med.REC.1395.41). Informed consent was obtained from each participant.

Results

Of the 932 recruited subjects, 496 (53.2%) were male and 436 (46.8%) were female. The range of participants' age was between 11 to 83 yr old and the mean age of the subjects was 35.4 (±12.7) years. Most of the subjects (36.81%) were in the age group 21-30 yr old. Anti-hydatid cyst antibodies were detected in the sera of 37 out of 932 subjects, corresponding to a seroprevalence rate of 3.96%. From these, 20 (54.05%) were male and 17 (45.95%) were female. Seropositivity to hydatid cyst was more prevalent in the age group of 21-30 yr (43.2%), however, there were no associations between seropositivity to hydatid cyst and age of the participants. Moreover, no statistically significant difference was seen between seropositivity to hydatid cyst infection and gender of the participants (P>0.05). Residential areas and having contact with dogs had no significant influence on the risk of hydatid cyst seropositivity. Table 1 shows the demographic features and relative seropositivity to hydatid cyst in the studied subjects in seven cities of North Khorasan Province, northeast of Iran.

Discussion

Hydatid cyst is a common zoonotic disease in many countries around the world. According to the WHO report, Iran, Iraq, and Turkey are among the hyper endemic areas for hydatid cysts infection in the Middle East. In Iran, hydatid cyst is one of the most important helminthic parasitic diseases and infection with *E. granulosus* is very common in final hosts in different areas of the country. In Iran, the rate of *E. granulosus* infection in dogs varies from 2.2% up to 63.2% in different areas of the country. High prevalence of *E. granulosus* infection in dogs ensures contamination of soil and vegetables and ultimately transmission of the infection to humans. The overall annual cost of CE in Iran was estimated at US$232.3 million, including both direct and indirect costs.

Table 1: Demographic features and relative seropositivity to hydatid cyst among people living in seven cities of North Khorasan Province, Northeast of Iran

Characteristics	*Frequency*	*Percent*	*Positive for anti- hydatid cyst antibodies*		*P-value*
			Frequency (No.)	*Percent (%)*	
Sex					
Male	496	53.2	20	54.05	*P*>0.05
Female	436	46.8	17	45.95	
Age group (yr)					
<20	64	6.87	2	5.4	
21-30	343	36.81	16	43.25	
31-40	251	26.93	10	27.03	
41-50	136	14.59	7	18.92	*P*>0.05
51-60	91	9.76	2	5.4	
>60	47	5.04	0	0	
Residence					
Bojnurd	410	44	30	81.08	
Shirvan	147	15.8	3	8.1	
Maneh and Samal-ghan	49	5.3	0	0	
Faruj	76	8.2	4	10.82	*P*>0.05
Raz and Jargaran	76	8.2	0	0	
Garmeh	90	9.7	0	0	
Jajarm	84	9	0	0	
Contact to dog					
Yes	211	22.63	15	40.54	*P*>0.05
No	721	77.37	22	59.46	

The cost associated with human CE was estimated at US$93.39 million and the annual cost associated with CE in livestock was estimated at US$132 million (10).

The findings of the current study revealed a seroprevalence rate of 3.96% for CE in North Khorasan province in Iran. The rate is somewhat similar to those reported in other parts of the country in previous studies (7, 9, 18-19). However, the seroprevalence rate in the current study was higher than those reported in Ilam, Hamedan, Isfahan, Zanjan, East Azerbaijan, Ardabil, and Kurdistan in Iran, which ranged from 0.23% to 3.6% (5, 20-22).

The rate of infection in males and females were almost the same in our study. This indicated that both men and women are equally prone to *E. granulosus* infection. The reason for this is that dogs are roaming around in all areas and through contaminating the environment, causes human infection, regardless of gender. Most of the previous studies in Iran have not revealed a specific difference between the sexes and hydatid cyst infection (4, 5, 13, 23). In Arak, central Iran, the rate of infection in females (3.99%) was higher than males (2.26%), but there was no significant statistical difference between infection rate of males and females (15). In Chaharmahal and Bakhtiari Province, southwestern Iran, females had a higher rate of CE infection than males (5.1% in females and 4.4% in males), without any significant statistical difference (24). However, in Khorramabad, capital of Lorestan Province in western Iran, CE was reported to be more prevalent in males (60%) than females (40%) and the difference between infection rate of males and females was significant (25).

In our study, there was no significant difference between the rate of infection in different age groups and the disease was fairly prevalent in all age groups. This disease can infect all people of any age and all people are at risk of infection. A large percentage of the adult hydatid cyst results from childhood infection. The seroprevalence of hydatid cyst in children in a rural community in Fars province, southern Iran has been reported to be 6.7% (13). In our study, 5.4% of young people were found to be seropositive for CE, which is somewhat similar to the rate reported in children in southern Iran.

Our study was done in seven cities of North Khorasan Province including Bojnurd, Shirvan, Maneh and Samalghan, Faruj, Raz and Jargaran, Garmeh and Jajarm. Although the prevalence of CE infection in these cities was somewhat different, there was no statistically significant difference between infection rates in different cities, which indicates that the disease is fairly prevalent throughout the province.

Conclusion

CE is relatively prevalent throughout the North Khorasan Province. Rate of CE infection in this Province is fairly similar to the rate of infection in other parts of the country.

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.

Acknowledgements

The study was financially supported by the vice chancellor for research at North Khorasan University of Medical Sciences.

Conflict of interest

None.

References

1. Romig T, Deplazes P, Jenkins D, et al. (2017). Ecology and life cycle patterns of *Echinococcus* species. *Adv Parasitol,* 95:213-314.
2. Craig PS, Hegglin D, Lightowlers MW, et al (2017). Echinococcosis: Control and prevention. *Adv Parasitol,* 96:55-158.
3. Galeh TM, Spotin A, Mahami-Oskouei M, et al (2018). The seroprevalence rate and population genetic structure of human cystic echinococcosis in the Middle East: A systematic review and meta-analysis. *Int J Surg,* 51:39-48.
4. Sarkari B, Hosseini F, Abdolahi Khabisi S, Sedaghat F (2016). Seroprevalence of cystic echinococcosis in blood donors in Fars province, southern Iran. *Parasite Epidemiol Control,* 2:8-12.
5. Sarkari B, Sadjjadi SM, Beheshtian MM, Aghaee M, Sedaghat F (2010). Human cystic echinococcosis in Yasuj District in Southwest of Iran: an epidemiological study of seroprevalence and surgical cases over a ten-year period. *Zoonoses Public Health*, 57:146-50.
6. Shafiei R TS (2016). The seroprevalence of human cystic echinococcosis in Iran: A systematic review and meta-analysis study. *J Parasitol Res,* 2016:1425147.
7. Dabaghzadeh H, Bairami A, Kia EB, et al (2018). Seroprevalence of human cystic echinococcosis in Alborz Province, Central Iran in 2015. *Iran J Public Health,* 47:561-566.
8. Fallah Omrani V, Rouhani S, Kazemi B, et al (2017). Seroprevalence of IgG antibodies against *Echinococcus granulosus* by ELISA method using recombinant AgB in Lorestan Province, Western Iran. *Iran J Public Health*, 46:1132-1138.
9. Heidari Z, Mohebali M, Zarei Z, et al (2011). Seroepidemiological study of human hydatidosis in Meshkinshahr district, Ardabil province, Iran. *Iran J Parasitol,* 6:19-25.
10. Fasihi Harandi M, Budke CM, Rostami S (2012). The monetary burden of cystic echinococcosis in Iran. *PLoS Negl Trop Dis,* 6:e1915.
11. Sarkari B, Rezaei Z (2015). Immunodiagnosis of human hydatid disease: Where do we stand? *World J Methodol,* 5:185-95.
12. Savardashtaki A, Sarkari B, Arianfar F, Mostafavi-Pour Z (2017). Immunodiagnostic Value of *Echinococcus Granulosus* Recombinant B8/1

Subunit of Antigen B. *Iran J Immunol*, 14:111-122.
13. Sarkari B, Arefkhah N, Ghorbani F, et al (2019). Seroprevalence of Cystic Echinococcosis and related risk factors for infection among children in a rural community in Fars Province, Southern Iran. *Clin Epidemiol Glob Health*, 8:13-16.
14. Arbabi M HH (2006). Survey of echinococcosis and hydatidosis in Kashan region, central Iran. *Iran J Public Health,* 35:75-81.
15. Asghari M MM, Kia EB, Farahnak A, et al (2013). Seroepidemiology of Human Hydatidosis Using AgB-ELISA Test in Arak, Central Iran. *Iran J Public Health*, 42:391-6.
16. Dadkhah MA YM, Nader B (2011). Survey on hydatid cyst infection in Sarab city (Northwest of Iran) using epidemiological and seroepidemiological criteria. *J Anim Vet Adv*, 10:2099-2101.
17. Esmaeili N AM (2010). Seroepidemiology of hydatidosis among adult human at Kashan region, Iran in 2008. *Journal of Kashan University of Medical Sciences*, 13:321-6.
18. Garedaghi Y, Bahavarnia SR (2011). Seroepidemiology of human hydatidosis by ELISA method in East-Azarbaijan Province in Iran in Year 2009. *Iran J Epidemiol*, 7:25-9.
19. Shafiei R, Ghatee MA, Jafarzadeh F, Javanshir Z, Karamian M (2019). Genotyping and phylogenetic analysis of unusually located hydatid cysts isolated from humans in north-east Iran. *J Helminthol.* 23;94:e64.
20. Akhlaghi L MJ, Housaini A (2005). Observation on hydatid cyst Infection in Kurdistan Province (West of Iran) using epidemiological and seroepidemiological criteria. *Iran J Public Health,* 34:73-5.
21. Zariffard MR AN, Akhavizadegan MA, Motamedi GR (1999). Seroepidemiological survey of human hydatidosis in western parts of Iran. *Arch Razi Ins*, 50:71–75.
22. Aflaki A GF, Dalimi Asl A (2006). Seroepidemiological survey of hydatidosis by Dot- ELISA in Ilam Province. *Pathobiol Res*, 8:1-6.
23. Moshfe A, Sarkari B, Arefkhah N, et al (2019). Seroepidemiological study of cystic echinococcosis in nomadic communities in the southwest of Iran: A population-based study. *J Immunoassay Immunochem*, 40(2):183-192.
24. Yousefi Darani H AM, Karimi K, et al (2003). Seroepidemiology of hydatid cyst in Chaharmahal va Bakhtiari Province, Iran. *Iran J Public Health,* 32:31-3.
25. Zibaei M, Azargoon A, Ataie-Khorasgani M, et al (2013). The serological study of cystic echinococcosis and assessment of surgical cases during 5 years (2007-2011) in Khorram Abad, Iran. *Niger J Clin Pract,* 16:221-5.