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Original Article

Seroprevalence of Human Cystic Echinococcosis in Sanandaj City, Kurdistan Province, Western Iran

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ABSTRACT

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Background and Aims: *Echinococcus granulosus* (*E. granulosus*) is a cestode parasite that causes cystic hydatid disease in humans worldwide. Iran is one of the endemic regions for infection that indicate the importance and presence of infection in this country. Therefore, the current research aimed to characterize the seroprevalence of human cystic echinococcosis in Sanandaj city, Kurdistan province, western Iran.

Materials and methods: Totally, 500 serum samples were collected from patients referred to different health centers in Sanandaj city using cluster sampling in 2018-2019. All the sera were examined using the enzyme-linked immunosorbent assay test.

Results: The seroprevalence of human hydatidosis was reported at 2.2% by ELISA test in Sanandaj city. This rate was 9 (1.9%) in women and 2 (0.4) in men. The age group of 20-30 years old had the highest positivity rate (1.0%). Also, the subjects that consumed home slaughtered meat had the highest infection rate at 4 (0.8%). There was no significant difference regarding factors studied such as sex, education, residence, consumed water, keeping a dog, and the seropositivity.

Conclusions: Seroprevalence of human cystic echinococcosis in Sanandaj city is lower than the general prevalence in Iran. Our research team hopes to provide accurate data on the prevalence of hydatidosis in Sanandaj encourage more extensive research to help prevent this parasite in Iran and worldwide.

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Introduction

Echinococcosis or hydatidosis is a fetal and significant helminthic disease caused by the larval stage of *Echinococcus granulosus* (*E. granulosus*) [1]. The definitive hosts for *E. granulosus* are canids, and a variety of hosts, including humans can be the intermediate host of this parasite [2]. Infection is transmitted by the consumption of the parasite's egg in contaminated food, water, or soil and contact with dogs [3]. Hydatid cysts can affect different parts of the body, and cysts usually form in the liver (50-70%), lungs (20-30%), and in other organs (spleen, brain, kidneys, peritoneal cavity, muscle, bone, and heart) (10%) [4]. Depending on the size of the cyst and its anatomical location, different clinical symptoms develop so that many patients may remain asymptomatic for years or never develop symptoms [5]. When a hydatid cyst occupies an organ, the resulting pressure on the surrounding tissues causes clinical symptoms. Anaphylactic shock and secondary cystic echinococcosis are significant complications of cyst rupture and discharge of their contents [6, 7]. The highest rate of this parasitic disease is reported in the east and south of Europe, the Middle East, Latin America, Africa, and the Mediterranean coasts, including Iran [8]. It is estimated that up to more than one million people are infected with this parasite globally each year, and in 2015, it resulted in 19300 death cases. Echinococcosis causes heavy economic losses in livestock about 3 billion dollars and the cost of treating patients with echinococcosis [9]. Treatment of this disease requires a correct diagnosis because misdiagnosis or uncertain diagnosis can eventually lead to recurrence or metastasis, even after surgery. Serology and imaging tests are widely used for clinical diagnosis [10]. Therefore, the present study detects the seroprevalence of hydatidosis using an enzyme-linked immunosorbent assay (ELISA) test and determines the risk factors involved in spreading the disease in Sanandaj city, Kurdistan province, western Iran.

Materials and Methods

Study area

This cross-sectional study was conducted in Sanandaj city, Kurdistan province, Iran. This district has bounded by Iraq in the west, Hamedan province in the east, Kermanshah province in the south, and western Azerbaijan Province in the north. It is located in the center of the province, and the population was 412767 according to the last census in 2016. The main economic activity of the people is agriculture and the husbandry of cows and goats.

Estimating sample size

The sample size was determined based on how many samples should be selected to estimate the serum prevalence of hydatid cyst so that with 95% certainty, a maximum of d = 0.01 differs from the real value. Prevalence was considered based on previous studies (p = 0.05). Accordingly, using the following formula, the sample size was 458 people.

$$n = \frac{z^2 P \times (1-P)}{d^2} \qquad n = \frac{(1.96)^2 \times 0.05 \times 0.95}{(0.01)^2} = 458$$

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The statistician consulting determined the sample volume as 458, but overall, 500 people were enrolled in the study for more caution.

Serum samples

Serum samples were collected from 500 cases, including 95 males and 405 females referred to clinical and health centers in Sanandaj, Kurdistan province, Iran, using cluster sampling in 2018-2019. A questionnaire was filled out for each case, including age, sex, education, residence, consumed water, consumption of home slaughtered meat, and keeping a dog. Sera were stored in the refrigerator at -20 °C and then were sent to the Department of Medical Parasitology, School of Public Health, Tehran University of Medical Sciences, Iran. The Ethics Committee of Islamic Azad University, Sanandaj Branch, Kurdistan, Iran, approved the study, and consent was obtained from the participants who volunteered to donate blood samples.

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. Concisely, 100 ml of HCF was dialyzed overnight against 5 mm acetate buffer (pH = 5) at 4 °C. The HCF was centrifuged at 30000 g for 30 min, and the pellet was dissolved in phosphate buffer (pH=8). Saturated ammonium sulfate was used to remove the globulin from the HCF. Lastly, the purified HCF antigen was boiled in a water bath for 15 min. At this stage, heat-resistant group B antigens remain stable, and antigen 5, because it is heat-sensitive, is denatured and becomes insoluble. Then, the solution was centrifuged at 30000 g for 60 min to isolate antigen B(AgB). Finally, supernatant-soluble B antigen was collected. After filtration and addition of (NaN3)2, the protein was divided into 1.5 ml microtubes with a 1 mg/ml concentration, and the antigen was kept at -80 °C [11].

ELISA

As previously described, the IgG-ELISA test was performed in 96 well microplates (Nunc, Denmark) [12]. Microplate wells were coated overnight at 4 °C with 100 µl AgB (20 µg/ml) in 0.05 M bicarbonate buffer, pH = 9.6. The wells were washed three times in phosphate-buffered saline (PBS) plus 0.05% Tween 20 (PBS-T) and blocked with PBS-T containing 1% bovine serum albumin (BSA) for 2 h at 37 °C. The wells were washed, and sera were added at 1:500 dilutions in PBS-T, incubated at 37 °C for 30 min, and then washed as before. Antihuman IgG-HRP (Sigma Chemical Co., Poole, Dorset, United Kingdom) conjugates were added at 1: 7000 dilutions in PBS-T, and the microplate was incubated and washed as before. It was then developed in o-phenylenediamine dihydrochloride (OPD) substrate (5 mg 1, 2-phenylenediamine, 12.5 ml of 0.2 M citrate phosphate buffer pH = 5, 10 µl 30% H_2O_2). The absorbance was read at 492 nm after 10 min using an automatic microplate reader (State Fax® 2100, Awareness, USA). The cut-off was calculated as mean + 3 SD.

Statistical analysis

The data were analyzed using SPSS 16 and Espino 6 programs. For calculating the needed sample, we used the expected prevalence of 0.05% at a confidence level of 95%. A P-value less than 0.05 was considered significant.

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Results

Out of 500 collected sera, 11 (2.2%) samples were seropositive for *E. granulosus* by ELISA test in Sanandaj city. Descriptive characteristics are presented in Table 1. Of the 500 individuals referred to health centers, 95 (19%) were male, and 405 (81%) were female. The average age was 32.17 ± 42 years. Figure 1 shows the age distribution of the subjects. This study showed no linear relationship between sex, residence, education, consumed water, keeping a dog, and hydatid cyst seropositivity ($P > 0.05$). However, there was a significant association between hydatid cyst seropositivity and age groups and the consumption of home slaughtered meat ($p < 0.05$). Based on the outcomes of this research, the seropositivity rates of echinococcosis according to age groups was as follows: < 20 yr, 0.4%; 20-30 yr, 1%; 30-40 yr, 0.2%; 40-50 yr, 0.2%; 50-60 yr, 0.2%; and >60 yr, 0.2%. The age group of 20-30 years old had the highest positivity rate. People who ate home slaughtered meat showed 0.8% seropositivity.

Discussion

In this study, seroprevalence for human hydatidosis was detected using the ELISA test in Sanandaj city. Accordingly, the total prevalence of human hydatidosis was 2.2% (11 cases). Cystic echinococcosis is one of the emerging zoonotic and endemic diseases in many of the Middle East countries, including Iran. Considering the significant number of patients infected with hydatidosis in Iran, the long prepatent period of human hydatidosis, and the risk of death after consecutive surgeries, awareness of the infection rate in different parts of the country is necessary for control measures. Previous studies based on various serological examinations from different parts of the country displayed the difference in prevalence.

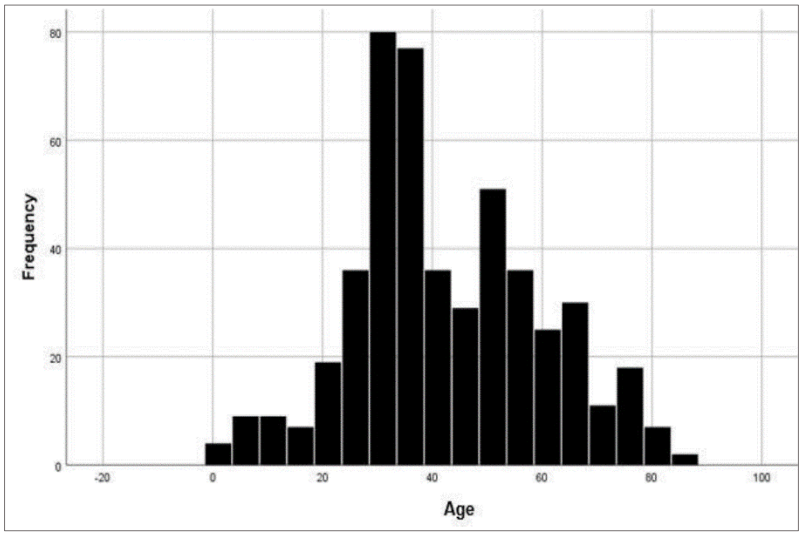

Fig.1. Age distribution of individuals in the study

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Table 1. Seroepidemiology of hydatidosis according to epidemiological factors observed in Sanandaj city, Kurdistan province, Iran

Characteristics	Number	Seroprevalence N (%)	P-value
Age groups (Years)	486	11 (2.3)	0.044
<20	38	2 (0.4)	
20-29	105	5 (1.0)	
30-39	119	1 (0.2)	
40-49	77	1 (0.2)	
50-60	64	1 (0.2)	
>60	83	1 (0.2)	
Sex	486	11 (2.3)	0.995
Female	398	9 (1.9)	
Male	88	2 (0.4)	
Residence	486	11 (2.3)	1
Urban	372	9 (1.9)	
Rural	114	2 (0.4)	
Education	500	11 (2.2)	0.109
Literate	331	10 (2)	
Illiterate	169	1 (0.2)	
Consumption of home slaughtered meat	485	10 (2.1)	0.026
I slaughter the sheep myself	10	0 (0)	
I eat slaughtered meat at home	19	4 (0.8)	
None	456	6 (1.2)	
Consumed water	479	4 (0.8)	1
Refined water	458	4 (0.8)	
Chlorinated and unrefined water	12	0 (0)	
Unrefined water	9	0 (0)	
Keeping a dog	479	11 (2.3)	1
Yes	42	1 (0.2)	
No	437	10 (2.1)	

According to the previous systematic review and meta-analysis study conducted by Shafiei et al. (2016), the estimated prevalence of cystic echinococcosis in Iran was 5 %, and the disease was most frequent among women and older patients. The highest prevalence was observed in the southwest and south of Iran [13]. In another systematic review and meta-analysis done by Khalkhali et al. (2017), the prevalence of human hydatidosis was 4.2% [14]. In a meta-analysis published in 2019, Mahmoudi et al. assessed the prevalence of echinococcosis in Iran using published studies. According to this meta-analysis study, the prevalence rate of echinococcosis in Iran has been reported as 5%. The prevalence of echinococcosis was significantly higher in the north and west of Iran, patients younger than 40 years of age, villagers, and nomads [15].

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In our study, a prevalence of 2.2% for human hydatidosis was detected in Sanandaj city, which is lower than the prevalence in the general population of Iran. A study conducted by Hadadian et al. (2005), with the aim of the seroepidemiological study of human hydatid cyst by ELISA method in Kurdistan province, showed that human hydatid cyst in Sanandaj and Divandarreh had a prevalence of 0.7% [16]. However, in the present study, the prevalence was higher. Comparing to previous similar studies conducted in Iran, it was lower than stated in Zanjan (3%) [17], Khuzestan in nomads (13.8%) [18], Chaharmahal and Bakhtiari (4.8%) [19], Tehran (5.9%) [20], Lorestan (2.6%) [21], Qaemshahr (2.73%) [22], and Golestan (2.34%) [23] Provinces, but higher than reported in Kashan (2.04%) [24], Ilam (1.2%) [25], Meshkinshahr (1.79%) [26], East-Azarbaijan (1.28%) [27], Isfahan (1.1%) [28], and Qom (1.6%) [29] provinces. Also, Akhlaqi et al. (2005) reported that the prevalence of hydatidosis by indirect immunofluorescence assay (IFA) test in sheep and cattle serum samples was 3.3% in Sanandaj and 9.5% in Divandarreh [30].

Our findings revealed that patients in the age group of 20-30 yr old were the main sufferers of hydatidosis, similar to the results of Harandi et al. and Sedaghat Gohar [20, 31]. Previous studies have reported the 10-19 yr old group as the highest infected age group in Zanjan [17], 60-80 yr old in Hamadan [32], 60-90 yr old in Meshkinshahr [26], 30-60 yr in Qom and Golestan [23, 29], and 40-49 yr old in Arak [33].

In the current study, 0.8% of the infected subjects had eaten home-slaughtered meat ($p < 0.05$). In this research, similar to other studies, no meaningful relationships were found between the prevalence of echinococcosis and sex ($p = 0.995$) [34-36], education ($p = 0.109$) [26, 34], residence ($p = 1$) [26, 29, 34, 35], consumed water ($p = 1$) [34], keeping a dog ($p = 1$) [34]. Dogs are an important source of *E. granulosus* infection. Close contact with dogs, especially in rural areas, as well as human-animal interactions can increase the serum prevalence of cystic echinococcosis [37, 38]. Dog owners, especially in rural areas, neglect precautions against infection such as feeding dogs, handling animals and their feces, and regular medical examinations [39]. Also, as a result of children and dogs playing, it is possible to transmit the parasite through randomly swallowed eggs [40]. Contrary to our results, Dabaghzadeh et al. reported that the rate of the disease was significantly higher in areas where dogs were higher in Alborz province, central Iran, in 2015 ($p < 0.05$) [34].

Also, a significant difference was seen according to literacy in Golestan ($p < 0.001$); illiterates had the highest rate of infection (3.72%). In the current study, the prevalence of cystic echinococcosis did not significantly differ based on education level. Rakhshanpour et al. reported that males were 2.5 times at higher risk of infection than females [29]. Nevertheless, our results did not show a significant difference between men and women.

Conclusion

Based on our research, the prevalence rate in Sanandaj city showed that hydatidosis is a

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potential risk to human health and is of great importance in this region due to high levels of husbandry and agriculture. Therefore, the authorities must take steps to prevent this disease, and increasing people's awareness of this disease may be considered one of the most important steps in fighting infection.

Conflicts of Interest

There is no conflict of interests to declare.

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