Seroprevalence of cystic echinococcosis in blood donors in Fars province, southern Iran

Bahador Sarkarib,c,⁎ Farshid Hosseinib Samaneh Abdolahi Khabisic, Farzaneh Sedaghatb

Basic Sciences in Infectious Diseases Research Center, Shiraz University of Medical Sciences, Shiraz, Iran
Department of Parasitology and Mycology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran
Department of Parasitology and Mycology, School of Medicine, Zahedan University of Medical Sciences, Zahedan, Iran

A R T I C L E   I N F O
Article history:
Received 18 November 2016
Received in revised form 6 December 2016
Accepted 6 December 2016
Available online 15 December 2016

Keywords:
Seroepidemiology
Cystic echinococcosis
ELISA
Fars province
Iran

A B S T R A C T
Cystic echinococcosis (CE) is one of the most important zoonotic diseases in different geographical areas of the world including Iran. The current study aimed to assess the seroprevalence of human cystic echinococcosis (CE) in healthy blood donors in Fars province, southern Iran. A total of 1068 serum samples were collected from blood donors from five blood service centers of Fars province in south of Iran. Antigen B was prepared from sheep hydatid cyst fluid and collected sera were evaluated for anti-hydatid cyst antibodies, using antigen-B ELISA. Demographic features of the participants were also recorded during the sample collection. Anti hydatid cyst antibodies were detected in sera of 60 out of 1068 blood donors corresponding to overall seroprevalence of 5.6% in this population. Rate of seroprevalence was 6.7% in females and 5.5% in males. The highest rate of infection (8.3%) was found in age group of higher than 50 years old. There were no significant differences between seropositivity to CE and sex, age or place of residence of the participants (P < 0.05). Findings of this study showed that the rate of CE infection in Fars province, southern Iran, is relatively high. Some of these seropositive cases may have active hydatid cyst in their tissues without overt sign or symptoms.

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1. Introduction

Cystic echinococcosis (CE) is a zoonotic infection of humans and domestic animals, caused by the larval stages of the dog tapeworm Echinococcus granulosus (Eckert and Deplazes, 2004). Dogs harbor the adults tapeworm and human acquire the infection through accidental ingestion of parasite's eggs. The disease is prevalent in different parts of Iran, and human cases are regularly reported from different regions of the country (Ahmadi and Badi, 2011; Dalimi et al., 2002; Elham et al., 2014; Sarkari et al., 2010; Ziaei et al., 2011). CE remains as one of the serious health problems in Iran and exerts heavy impact on the economy and social welfare of the people (Fasihi Harandi et al., 2012). Mean number of surgical CE cases per year for 2000–2009 in the country has been estimated at 1295 and the number of asymptomatic individuals living in the country estimated at 635,232 cases (Fasihi Harandi et al., 2012). The overall annual cost of CE, including both direct and indirect costs, in Iran has been estimated at US$232.3 million (Fasihi Harandi et al., 2012).

The seroprevalence of CE varies in different geographical regions of the world, ranging from 1 to higher that 15% in different areas. Blood donors in Iran are usually healthy people who voluntarily donate blood just to help those in dreadful need of
blood. The current study aimed to find out the seroprevalence of CE in a defined group, blood donors, in one of the largest provinces of Iran, Fars, in south part of the country.

2. Materials and methods

2.1. Study area

This cross-sectional study was conducted in Fars province which is one of the thirty-one provinces of Iran and known as the cultural capital of the country. It is located in the south of the country and its administrative center is Shiraz (Fig. 1). It has an area of 122,400 km². In 2011, this province had a population of 4.59 million people, of which 67.6% were registered as urban dwellers (urban/suburbs), 32.1% villagers (small town/rural), and 0.3% as nomad tribes.

2.2. Serum samples

After getting approval from the ethics committee of Shiraz University of Medical Sciences, blood samples were taken from a total of 1068 blood donors in five cities of Fars province of Iran (Darab, Firooz Abad, Kazeroon, Jahrom and Noor Abad) in 2013–2014 (Fig. 2). Samples were randomly collected from the subjects who donated the blood in blood service centers in each of five cities. Sera were stored at −20 °C, until use. Sample size was valued based on the population of each city. Demographic characteristic of participants were noted during sample collection.

2.3. Preparation of antigen

Hydatid cyst fluid (HCF) was aspirated from hydatid cysts, obtained from livers of sheep slaughtered at the local abattoir. Antigen B (AgB) was purified and extracted from hydatid cyst fluid as previously described (Sarkari et al., 2007; Sadjjadi et al., 2009). Briefly, 50 ml of HCF were dialyzed overnight against acetate buffer (5 mM, pH 5) at 4 °C. To remove the albumin, the sample was centrifuged at 50,000 g for 30 min and the pellet was dissolved in 0.2 M phosphate buffer (pH 8). Saturated ammonium sulfate was used to eliminate the globulin from the sample. Lastly, the sample was boiled for 15 min and centrifuged at 50000 g for 60 min to isolate antigen B from other antigens.

2.4. Antigen-B ELISA

Sera samples were assessed for antibodies to hydatid cyst by antigen-B ELISA (Sadjjadi et al., 2007). Flat-bottom 96-well microplates (Nunc, Denmark) were coated, overnight, with 100 μl of AgB (5 μg/ml) in 0.05 M bicarbonate buffer, pH 9.6. Wells were washed three times in PBS plus 0.05% Tween 20 (PBS–T) and blocked with PBS–T containing 3% skimmed milk for 30 min at 37 °C. Sera were added, at 1:100 dilutions in PBS-T, and the plate was incubated at room temperature for 2 h. The plates were washed as before. Horseradish peroxidase (HRPO) conjugated anti-human antibodies (Sigma Chemical Co., Poole, Dorset, United Kingdom) was added, at 1: 4000 dilutions in PBS–T, and the microplate was incubated for 1 h and washed as before. The reaction was
then developed in OPD substrate (5 mg o-phenylenediamine, 12.5 ml of 0.2 M citrate phosphate buffer pH 5, 10 μl of 30% H$_2$O$_2$).
The absorbance was read at 492 nm after 30 min, using an automatic microplate reader (BIO-TEK®, ELx800, USA). The cut-off point was set as 3 SD above the mean of controls. Positive and negative controls were included in each run of ELISA.

3. Data analysis

All data were analyzed using SPSS software ver. 16 (Chicago, USA), using chi-squared and Fisher exact tests to compare the seroprevalence values relative to the features of the subjects. P value $<0.05$ was considered as significant.

4. Results

Subjects of the study were 1008 males (94.4%) and 60 females (5.6%), from 5 cities of Fars province, including Darab, Firoozabad, Jahrom, Kazeroon and Noorabad. Of all the subjects, 327 cases were aged 21–30 years old and 304 cases were aged 31–40 years old. Anti-hydatid cyst antibodies were detected in 60 out of 1068 cases (5.6%).

Rate of seropositivity was 6.7% in females (4 cases out of 60 cases) and 5.5% in males (56 cases out of 1008 cases). The difference between sex and seropositivity to hydatid cyst was not statistically significant (P $>0.05$).

The highest rate of seropositivity to hydatid cyst in blood donors in this study was found in Firoozabad city while the lowest rate was found in Darab city. No statistically significant difference was found between seroprevalence rate in different cities (P $>0.05$). The highest rate of seropositivity was found in age group of higher than 50 years old (Table 1).

Although different rates of seropositivity were seen in different age groups, yet the differences between the age groups and seropositivity to hydatid cyst were not statistically significant (P $>0.05$). Regarding the occupation of the subjects, most of

| Age (year) | Anti-hydatid cyst antibodies number (percent) |  |
|-----------|-----------------------------------------------|---|
|           | Positive                                      | Negative | Total |
| 10–20     | 0 (0)                                         | 20 (100) | 20    |
| 21–30     | 18 (5.5)                                      | 309 (94.5) | 327 |
| 31–40     | 15 (4.9)                                      | 289 (95.1) | 304 |
| 41–50     | 14 (5.4)                                      | 245 (94.6) | 259 |
| >50       | 12 (8.3)                                      | 131 (91.7) | 143 |
| Total     | 59 (5.6)                                      | 994 (94.4) | 1053 |
them (46%) were self-employed, while 26% were employee, 7% were students, 5% were farmer and 16% were unemployed. No significant difference was found between occupation of the participants and seropositivity to hydatid cyst ($P > 0.05$).

5. Discussion

Human CE remains as a significant health problem in many of developing countries. The current study was done on 1068 blood donors in southern Iran. Since the target population of the study was blood donors, and in view of the fact that blood donors are mostly males and also are from specific age groups, therefore, the seroprevalence of CE reported in the study does not necessary shows the seroprevalence of hydatid cyst in the community. Most of these seropositive cases are asymptomatic individuals with hydatid cyst, but without apparent signs or symptoms. Blood donors may be infected by a few of parasitic diseases which may be transmitted to the recipient through transfusion (Sarkari et al., 2014, 2015). However there is no risk of transmission of CE through blood transfusion.

According to data obtained in this study, the rate of seroprevalence in the studied population is 5.6%. Seroprevalence was assessed by an AgB-ELISA which is one of the most sensitive and specific test in serological diagnosis of CE (Sadjadi et al., 2007; Rahimi et al., 2011; Mohammadzadeh et al., 2012; Sarkari and Rezaei, 2015). Previous serological studies from various parts of the country showed different rate of seroprevalence ranged from 1 to more that 7% (Cetinkaya et al., 2005; Dalimi et al., 2012; Sarkari et al., 2010; Zibaei et al., 2013).

Different serological studies have been done regarding the seroprevalence of CE in different geographical regions of the word (Abu-Hasan et al., 2002; Cetinkaya et al., 2005; Heidari et al., 2011; Qaqish et al., 2003; Sarkari et al., 2010; Yang et al., 2008). Using antigen B-ELISA, same as the current study, a seroprevalence study in Khorram Abad, Iran, revealed anti-CE antibodies in 15.4% of the 617 participants (Zibaei et al., 2013). In Yousefi et al. study on 1000 blood donor in Chaharmahal and Bakhtiari Province, 33 out of 1000 (3.3%) of blood donors were seropositive for hydatid cyst (Yousef and Avijegan, 2001). The rate of seroprevalence of CE in our study is almost similar to the rate of infection in other parts of Iran (Heidari et al., 2011; Yousef and Avijegan, 2001; Zibaei et al., 2013).

In a study in Turkey, anti-CE antibodies were investigated in the serum samples of CE suspected patients and the results showed that 439 out of 2921 cases (15.03%) were seropositive (Beyhan et al., 2015).

In our study no significant statistical difference was found between the rate of seropositivity to hydatid cyst in males and females. In Asghari et al. study in Arak, central Iran, the rate of CE in females was higher (3.99%) than males (2.26%), but again the differences were not statistically significant (Asghari et al., 2013). Similarly in Yousefi et al. study in Chaharmahal and Bakhtiari Province, Iran, females had relatively higher rate of CE infection but the differences between males and females were not statistically significant (Yousef and Avijegan, 2001).

Our study was done in five cities of Fars province. The rate of infection was almost similar in the five cities, which indicates that the disease is fairly prevalent throughout the province. Taken together, findings of this study showed that CE, as an important zoonotic disease, is relatively prevalent throughout the Fars province in southern Iran. Some of these apparently healthy people may have tissue hydatid cyst, without having sign or symptoms.

Conflict of interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgements

The results described in this paper were part of MD thesis of Farshid Hosseini. The study was financially supported by the office of vice-chancellor for research of Shiraz University of Medical Sciences (Grant No. 92-01-01-5964).

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