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Original Article

Seroepidemiology of Human Hydatidosis in Golestan Province, Iran

*M Baharsefat, J Massoud, I Mobedi, A Farahnak, *MB Rokni*

Dept. of Medical Parasitology and Mycology, School of Public Health, Medical Sciences /Tehran University, Tehran, Iran

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Abstract

Background: Hydatidosis is one of the most prevalent zoonotic diseases worldwide. So far no survey was conducted to determine the rate of human hydatidosis in Golestan Province, so using IFA and ELISA tests the prevalence of this disease was detected in patients referred to health centers in this province.

Methods: Totally 1024 serum samples were collected from patients referred to different health centers in 4 cities of Gloestan Province including Gorgan, Gonbad kawoos, Aliabad Katool and Kordkoy. All the sera were examined using IFA and ELISA tests.

Results: Twenty four cases (2.34%) were positive for hydatidosis in Golestan Province using IFA, whereas 22 cases (2.15%) showed positivity using ELISA. Gorgan, Gonbadkaoos, Aliabad Katool and Kordkoy demonstrated the rate of positivity as 1.41%, 2.40%, 5.36% and 2.30%, respectively, but no significant difference was seen. As to positivity, there was no significant difference between age groups, sex, different cities and rural or urban life, but a significant different was seen according to job and literacy ($P< 0.001$). According to Job and literacy, housewives and illiterates had the highest rate of infection as 3.67% and 3.72%, respectively. As regards residency, urban life showed no significant difference with rural life (2.47% vs. 2.45%). Age group of 40-49 years old had the highest rate of positivity (3.95%). Females were more infected than males (3.16% vs. 1.93%).

Conclusion: The rate of prevalence in this province shows somehow a resemblance with the other cities in Iran. Considering the lifestyle in this province a complementary study is suggested in all related cities.

Keywords: *Seroepidemiology, Human hydatidosis, IFA, ELISA, Iran*

Introduction

Hydatidosis is one of the most prevalent zoonotic diseases in the world, causing major economical and healthy problems. The agent of the disease is *Echinococcus granulosus*, a parasite of cestodes, having its final host as dog and a variety of hosts including human as intermediate hosts. This parasite is cosmopolitan and posses the second rank in consideration of helminthic diseases significance (1). The highest rate of infection is reported form east and south of Europe, Mediterranean coasts, Middle East, Latin America and Africa, mostly in rural districts (2).

Larval cysts or hydatid cysts can be found in many tissues, most often in the liver, lung; mediastinum, peritoneum, and nearly every site of the body. Main clinical symptoms in humans include liver dysfunction, lung problems, ascites, abdominal pain, hepatomegaly, splenomegaly, central nervous system disorders (1). Cystic echinococcosis is considered endemic in the entire Mediterranean zone including all countries from the Middle East. Alveolar echinococcosis is less prevalent (3).

Both causative agents of the disease are reported in Iran and hydatid disease is responsible for approximately 1% of admission to surgical wards, a figure which has increased remarkably recently due to increasing number of Afghani refugees residing in Iran (4, 5).

In Iran due to encompassing various climatic conditions, the rate of the disease is diverse in various parts. Because of washing the parasites'

20 ***Corresponding author:** Te/Fax: +98 21 88950184, E-mail: mbrokni@gmail.com

eggs in rainy regions the rate is lower. The most infected districts are the domains of Alburz and Zagros Mountains where having a high rate of ruminants and the main carrier of people is animal husbandry (2).

Considering that no previous study was conducted in Golestan Province to detect the seroprevalance of hydatidosis, this survey was conducted.

Materials and Methods

Serum samples were collected from 1024 cases refereed to clinical and health centers, as well as private and governmental laboratories in the cities of Golestan Province including Gorgan, Kordkoy, Aliabad Katool and Gonbad Kawoos. A questioner was filled out for each case including various factors such as age, sex, job, locality, literacy and so on. 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 IFA and ELISA.

All data were analyzed using SPSS software ver. 10.

Antigen

Hydatid fluid (CHF Ag) was aspirated from hydatid cysts obtained from livers and lungs of sheep slaughtered at the local abattoir. The hydatid fluid was centrifuged at 1000×g for 15 min and supernatant was dialyzed against extensive distilled water overnight at 4 °C. The dialysate was then filtered, lyophilized and stored at -20 °C until use in ELISA test. Collected protoscolices were utilized as antigen in IFA test.

IFA test

IFA was done as described previously (6). In brief, prepared protoscolices were incubated with serial dilutions of test serum as 1/10 dilution, washed, then incubated with 100 µl fluorescein-ated goat anti-human IgG, rewashed and observed for cuticular fluorescence.

ELISA test

ELISA test was performed in 96 well microplates (Nunc, Denmark) as previously described (7), with some modifications. Microplate wells were coated overnight at 4 °C with 100 µl CHFAg (2 µg/ml) in 0.05 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 1:250 dilutions in PBS-T, incubated at 37 °C for 1 h then washed as before. Antihuman IgG-HRP conjugates were added at 1: 2000 dilutions in PBS-T and the microplate incubated and washed as before. This was then developed in 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). Cut- off was calculated as X + 2 SD.

Results

Twenty four cases (2.34%) were positive for human hydatidosis in Golestan Province using IFA, whereas 22 cases (2.15%) showed positivity using ELISA. Cut-off was calculated as 0.241. Fig. 1 shows scatter diagram for OD absorbances in ELISA test.

Gorgan, Gonbadkawoos, Aliabad Katool and Kordkoy demonstrated the rate of positivity as 1.41%, 2.40%, 5.36% and 2.30%, respectively, but no significant difference was seen.

There was no significant difference between age groups, sex, different cities and rural or urban life, but a significant different was seen according to job and literacy ($P< 0.001$). Housewives had the highest rate of infection (3.67%) followed by farmers, employees, and free jobs as 2.1%, 0.58% and 2.03%, respectively, which showed a significant difference ($P< 0.001$). According to literacy, illiterates had the highest rate on infection (3.72%). As to residency, urban life sowed no significant difference with rural life (2.47% vs. 2.45%). Age group of 40-49 yr old had the highest rate of positivity (3.95%). Detailed characteristics of cases according to age group are shown in Table 1. Females were more infected than males (3.16% vs. 1.93%), but the difference was not significant.

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

Age group (yr)	Total no.	Positivity (no.)
0_9	16	0
10_19	121	0
20_29	227	4
30_39	243	6
40_49	177	7
50_59	120	6
60_69	68	1
70<	52	0
Total	1024	24

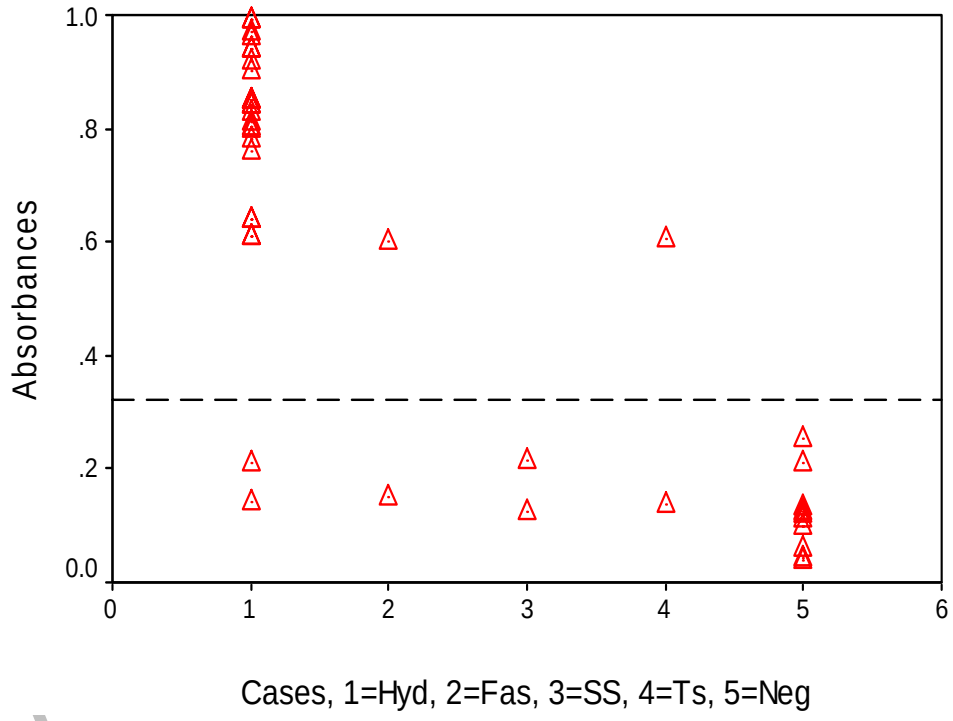

Fig. 1: Analysis of sera from patients with various single infections by IgG-ELISA. Serum samples obtained from patients with hydatidosis (Hyd), fasciolosis (Fas), strongyloidiasis (SS), trichostronglosis (Ts) and normal controls (Neg).

Discussion

Twenty four cases (2.34%) in this study were positive for human hydatidosis using IFA, whereas 22 cases (2.15%) showed positivity using ELISA. Comparing various studies conducted in Iran, more or less, a similar result has been gained. In Urmia, west-Azerbaijan Province, 202 patients with hydatid cyst were detected from 1991 to 2001. 65.85% of cases were female, whilst farmers and housewives encompassed the highest rate as regards career (8). In a seroepidemiological study in Zanjan, center of Iran, 3% of studied cases were positive for hydatidosis using indi-

rect ELISA (9). The highest rate belonged to 10-19 yr old. Positive rate in males and females was 2.7% and 3.2%, respectively (9). Some miscellaneous studies reported the prevalence of hydatidosis as 5.9% in Shahryar via IFA technique (10), and 4.8% in Chaharmahal va Bakhtyari Province using counter immunoelectrophoresis (11). Also in Varamin area in south of Tehran 68 out of 700 serum samples (9.7%) were positive by IFA test (12). In Sanandaj area, 3.3% and in Divandareh, 9.5% of serum samples were positive for human hydatidosis (13).

Dogs play an important role in transmitting the disease in Iran. In a widespread study conducted in 13 provinces of Iran, the prevalence of *E. granulosus* in sheepdogs was detected as 27.17% (14). In addition, in west part of Iran 20% infection rate in stray dogs has been reported (15). Nine stray dogs (44%) autopsied in Sanandaj were positive for Echinococcosis (13). Besides this factor, consuming row vegetables and contact with soil also have a key factor in acquiring the disease. All these factors can be seen in different cities of Golestan Province.

Females were more infected than males (3.16% vs. 1.93%). Some different studies in Iran showed a higher rate of infection to hydatid disease in females than males (16, 17). More contact with soil and vegetable impose an important factor to cause more infectivity in females.

Age group of 40-49 yr old had the highest rate of positivity (3.95%). Arbabi *et al.* reported the highest rate of infection with hydatidosis in Hamadan in age group of 60-80 yr (18), while Hoseini *et al.* reported this in Kurdistan Province in 20-40 yr old (19). As in accordance with the other studies conducted here and there, the rate of infection demonstrates itself in housewives more than other jobs (8, 17, 20). In this study illiterates showed the highest rate of infection which was in accordance with Sedaghatgohar report in Shahryar district (10).

Residency could not demonstrate a significant difference in this study but in earlier studies rural life showed significant difference with urban life (8, 17, 20).

Conclusively as it was expected, in Golestan Province infection with hydatidosis can be found, so the preventive measures as sated by authorities in this field must be taken into account, the most important of which, is teaching health measures to all people.

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