Tehran University of Medical Sciences Publication
http:// tums.ac.ir

Iranian J Parasitol

Open access Journal at
http:// ijpa.tums.ac.ir

Iranian Society of Parasitology
http:// isp.tums.ac.ir

Original Article

Seroprevalence of Human Fascioliasis in Meshkin-Shahr District, Ardabil Province, Northwestern Iran in 2012

Samieh ASADIAN [1], Mehdi MOHEBALI [1,2], Mahmod MAHMOUDI [3], Eshrat Beigom KIA [1,2], Zahra HEIDARI [1], Majid ASGARI [1], Mojgan ARYAEIPOUR [1], Sepideh MORADI [1],*Mohammad Bagher ROKNI [1,2]

1. *Dept. of Medical Parasitology and Mycology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran*
2. *Center for Research of Endemic Parasites of Iran (CREPI), Tehran University of Medical Sciences, Tehran, Iran*
3. *Dept. of Epidemiology and Biostatistics, School of Public Health, Tehran University of Medical Sciences, Iran*

Received 24 Aug 2013
Accepted 10 Oct 2013

Keywords
Seroprevalence,
Human,
Fasciolosis,
ELISA,
Iran

***Correspondence**
Email:
roknimoh@tums.ac.ir

Abstract

Background: The aim of this study was to conduct a seroprevalence survey in Meshkin-Shahr, Ardabil Province, north western Iran to detect the rate of human fascioliasis in the city and nearby villages. Literature shows that no such study has been conducted so far.

Methods: Overall, 458 serum samples were collected by randomized cluster sampling method from 153 males and 305 females referred to different health centers of the region after recalling by staff in those centers in 2012. All cases filled out a questionnaire and an informed consent. Sera were analyzed using indirect-ELISA test. Ten µg /ml antigens (Liver Fluke Homogenate), serum dilutions of 1:500 and conjugate anti-human coombs with 1:10000 dilutions were utilized to perform the test. Data analysis was conducted using SPSS software ver. 18.

Results: Nine cases (1.96%) were positive for fascioliasis by ELISA test. The seroprevalence of fascioliasis among females was 1.63% and 2.6% in males. There was no significant difference as regards age groups, sex, job, residency, literacy and consuming row vegetable. According to job, unemployment subjects had the highest rate of infection as 5.9%. The seroprevalence of infection was 1.52% in illiterate people. As for residency, urban life showed no significant difference with rural life (2.4% vs. 1.42). Age group of 40-49 yr old, with 3.3% seropositivity had the highest rate.

Conclusion: Obtained seroprevalence of fascioliasis shows immediate attention of health authorities to the diseases in the area. The adjacent of Ardabil Province to endemic areas of fasciolosis accentuates this attention.

Introduction

Fasciolosis, caused by the parasites of *Fasciola* spp., is regarded as one of the important parasitic disease among the neglected tropical diseases. Although not long time ago, it was considered typically as a veterinary issue, but at present ranks among the highest significant food borne diseases by WHO, FAO and many researchers (1). Estimates of human infection in Asia and Africa vary from 2.4-17 million people (2). People at risk are estimated 91.1×10^6 (3). It was estimated in 2005 that about 56.2 million people were infected with food-borne trematodes, 7·9 million had severe sequelae, and the global burden was 665,352 DALYs, of which a most important share was attributed to fasciolosis (4).

In Iran, until 1989, human fascioliasis was sporadic in Iran with several case reports of human infections but after occurring two important outbreaks of fasciolosis in 1989 and 1999, during which the world largest ever outbreaks occurred in Gilan Province, northern Iran and affected more than 10000 people in each of which (5-8) the feature was changed and its importance became obvious.

Following the occurrence of the various features of fasciolosis, the need for verifying the prevalence in different areas of Iran was palpable. Indeed lots of challenges on the different aspects of the disease, including treatment, diagnosis, following up, incidence, prevalence etc now have been arisen which caused the researcher of the country to focus more on this disease.

The present study was based on the aforementioned need to verify the prevalence of fasciolosis in one of the most talented areas of Iran in the context of having human fasciolosis, i.e., Ardabil Provinces, northwestern Iran. We tried to detect the rate of prevalences and different epidemiological aspects of human fasciolosis in Meshkin-Shahr in that province because of its nearby locality to most critical provinces of fasciolosis and its ecological situation.

Materials and Methods

Samples

Meshkin-Shahr district is located in the north-west of Iran. It covers an area of approximately 1530 km^2 and its population is estimated to be 237 585, among whom 29.7% are settled in urban areas and 70.3% live in 323 rural areas (https://en.wikipedia.org/-wiki/Meshginshahr). The distribution of the population in terms of urban and rural residency is equal (Fig. 1).

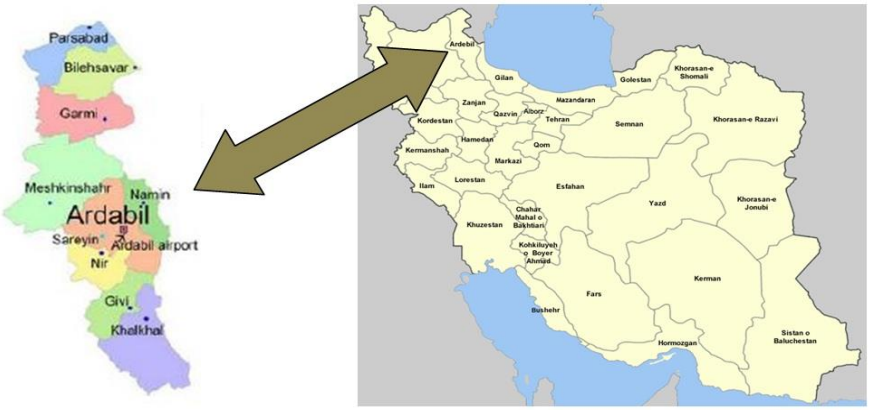

Fig. 1: Situation of Ardabil Province in Iran

Overall, 458 serum samples were collected by randomized cluster sampling method from 153 males and 305 females referred to different health centers of the region after recalling by staff in those centers in 2012. Therefor the sampling was such that randomly a representative sample of the society was surveyed for the disease. Sera were collected from 4 rural points of High, Koojengh, Youssef Kahn, and Naghdi Olia and four regions of urban areas as Gharehdarvish, Sayyedabbad, Farhangian and Parasil.

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.

All cases filled out a questionnaire and an informed consent. The Ethical Committee of Tehran University of Medical Sciences approved the study.

Preparation of antigens

Adults of *Fasciola hepatica* were obtained from infected sheep livers collected from local abattoirs. Liver fluke homogenate (LFH) antigen was prepared by homogenizing adult worms in 0.045 M PBS/pH 7.2 using electrical homogenizator (Edmund Buhler Co., model Homo 4/A mituhr) followed by sonication (Tommy Seiko model UP-200P, Tokyo), and then centrifugation at 15000g at 4 °C for 30 min.

ELISA

The immunodiagnostic assay was performed as previously described (9) with some modifications. Ten µg /ml antigens (LFH), serum dilutions of 1:500 and conjugate anti-human coombs with 1:10000 dilutions were utilized to perform the test.

Statistical analysis

The cut-off value was calculated as means plus 3.0 standard deviation OD value of the healthy group sera. Accordingly, higher OD values were considered as positive and vice versa.

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

Results

Nine cases (1.96%) or 196/10000 were positive for fascioliasis by ELISA test. Figure 2 shows the distribution of OD absorbance in subjects and healthy control cases. Cut-off was calculated as 0.32.

The seroprevalence of fascioliasis among females was 1.63% and 2.6% in males. There was no significant difference as regards age groups, sex, job, residency, literacy and consuming row vegetable. Age wise prevalence showed 3.3% as the highest rate in age group of 40-49 yr old (Table 2). According to job, unemployment subjects had the highest rate of infection as 5.88%, followed by 4.08% in students, 3.6% in farmers and 1.18% in housewives. The seroprevalence of infection was 1.52% in illiterate people, 9% in subjects with BSc, 2.6% with diploma, 2% with less than diploma and 0% with doctoral documents but the difference was not significant. As for residency, urban life showed no significant difference with rural life (2.4% vs. 1.42%).

Table 1: Age wise prevalence of positive cases among subjects examined from Meshkinshahr, Ardabil Province, Iran

Age group (yr)	Total No.	Seropositive casesN (%)
0-9	40	1 (2.5)
10-19	37	1 (2.7)
20-29	59	0
30-39	90	1 (1.1)
40-49	90	3 (3.3)
50-59	74	1 (1.3)
>60	68	2 (2.9)
458	458	9 (1.96)

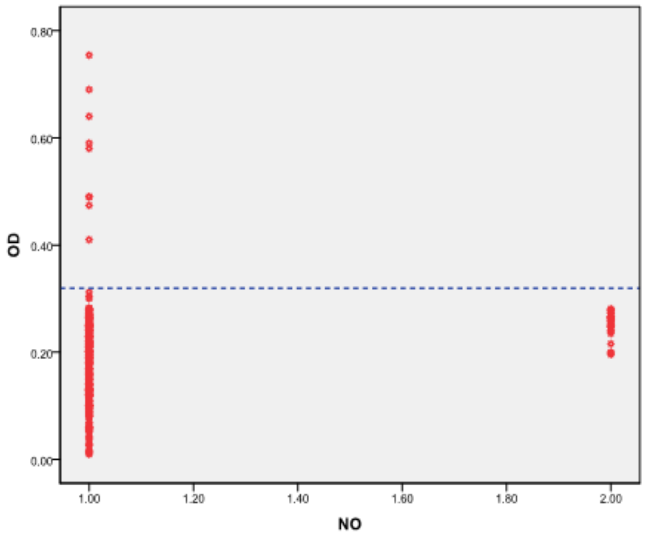

Fig. 2: Analysis of sera from subjects and normal controls from Meshkinshahr, Ardabil Province, Iran by IgG-ELISA. Serum samples obtained from subjects (458, Lanes 1), and normal controls (30, Lanes 2)

Table 2: Prevalence surveys conducted in Iran from 1991 to 2012

Date	Location (Province)	Prevalence (number tested)	The highest infected age range (yr)	Male: Female infections	Diagnostic method	Reference
1991	Gilan	50 (452)	10-19	73/153	Serology (ELISA)	(6)
2012	Gilan Province	1.2 (1283)	> 20	8/7	Serology	(13)
2012	Gilan Province	0.4 (1283)	> 20	3/2	Coprology	(13)
2011	Kohgyluyeh and Boyreahmad Province	1.8 (1000)	41-50	9/9	Serology (ELISA)	(23)
2012	Ilam	0.7 (600)	30-50	1/3	Serology (ELISA)	(24)

Discussion

In this study we could detect the seroprevalence of human fasciolosis as 1.96% in Meshkinshahr, Ardabil Province. This study follows the continuous studies conducted in Iran to determine the situation of human fasciolosis. This province has required criteria as regards involving with parasitic diseases (10-12). Among these criteria ecological situation, adjacent to endemic areas of fasciolosis, culture, food habitation, etc might be named. The rate of 1.96% seropositivity shows that further monitoring is needed to detect the real situation of the disease in the area. Similar to other serological studies it is not possible to judge the real rate of infection and further diagnostic methods such as stool exam are necessary. Although in Iran, two outbreaks of human fasciolosis during the years of 1989 and 1999 have been occurred called the world's largest ever outbreaks (5, 6), Forghan-Parast and Ashrafi (7) but recent surveys reveal prevalence of 0.4% and 1.2% using coprological and serological methods, respectively, con-

firming a hypoendemic situation (13). World Health Organization (WHO) included Iran among six countries which are known to have a serious fasciolosis problem (14). Table 2 shows the rate of human fasciolosis through different studies conducted in Iran.

In northern endemic areas of Iran, numerous irrigation canals, agricultural crop traditions (mainly rice), temperatures higher than 20 ºC, high rainfall (1300-1800 mm annually), and short dry seasons, favor fasciolosis transmission and lymnaeid populations (15). Fuentes et al. have demonstrated by mathematical modeling the impact of annual rainfall in determining the level of transmission in endemic areas (16). All these data are witness to necessity of monitoring fasciolosis in Iran.

In our study the highest rate of infection was seen in age group of 40-49 yr old as 3.3%. In the outbreaks in Gilan and Kermanshah the highest number of infected individuals was seen in the 10-29 year and in 10-19 year age groups, respectively. In comparison, in non-epidemic situations, the numbers of infected cases were higher in older (> 20 year) age groups (13, 17). Generally, the pattern of age wise prevalence shows that in Iran, adults are infected more than children (10, 15).

In comparison to other countries of the world especially those of nearby region, the population at risk of human fasciolosis in Egypt is considered to be 27 million (18) and the prevalence ranging from 2-11% (19). Four human cases of fasciolosis have been reported from Algeria (20). The 0.31% prevalence of human fasciolosis has been reported in Lahor, based on fecal exams performed in 2003-2005 (21). In Yemen human fasciolosis was indentified in 0.5% of 37,000 people during the period 1980-1982; *Fasciola* ova were found 185 cases (22). It shows that fasciolosis is a disease of immediate topic for consideration not only in Iran but in all other countries.

Conclusion

Obtained seroprevalence of fascioliasis in Meshkin-Sshahr shows that a global study to detect the rate of fasciolosis is necessary in Iran. Health authorities should pay attention to prevent of spreading the disease through public education, mass treatment, ecological consideration etc. The ease of sending local foods from endemic areas and frequency of intermediate hosts has prepared a situation which increases the possibility of involving with the disease.

Acknowledgements

The study was financially supported by Deputy of Research. Tehran University of Medical Sciences through grant No. 19838. The field activities were carried out at the Meshkin-Shahr Health Station, National Institute of Health Research (NIHR), Iran, which is highly appreciated. The study was conducted as an MSPH thesis. The authors declare that there is no conflict of interest.

References

1. Mas-Coma S, Bargues M. Human liver flukes: A review. Res Rev Parasitol. 1997;57:145-218.
2. Mas-Coma S, Bargues MD, Valero MA. Fascioliasis and other plant-borne trematode zoonoses. Int J Parasitol. 2005;35:1255-1278.
3. Keiser J, Utzinger J. Emerging foodborne trematodiasis. Emerging Infectious Diseases. 2005;11:1507.
4. Fürst T, Keiser J, Utzinger J. Global burden of human food-borne trematodiasis: A systematic review and meta-analysis. The Lancet Infectious Diseases. 2012;12:210-221.
5. Massoud J. Fasciolosis outbreak in man and drug test (Triclabandazole) in caspian littoral, northern part of Iran. Bull Soc France Parasitol. 1989;8:438.
6. Assmar M, Milaninia A, Amir-Khani A, Yadegari D, Forghan-Parast K, Nahravanian H, Piazak N, Esmayli A, Hovanesian A, Valadkhani Z. Seroepidemiological investigation of fascioliasis in northern Iran.

Medical Journal of the Islamic Republic of Iran. 1991;5:23-27.
7. Forghan-Parast K, Ashrafi K. Comparison of the formalin-ether and kato-katz in the parasitological diagnosis of human fascioliasis. J Med Fac Gilan Univ Med Sci Iran. 2001;9:1-6. [In Persian].
8. Rokni MB. The present status of human helminthic diseases in Iran. Ann Trop Med Parasitol. 2008;102:283-295.
9. Rokni MB, Massoud J, O'Neill SM, Parkinson M, Dalton JP. Diagnosis of human fasciolosis in the Gilan Province of northern Iran: Application of cathepsin L-ELISA. Diagn Microbiol Infect Dis. 2002;44:175-179.
10. Rokni MB. The present status of human helminthic diseases in Iran. Ann Trop Med Parasitol. 2008;102:283-295.
11. Mowlavi G, Mamishi S, Rokni M, Mobedi I, Gharaguzlo M, Ashrafi K, Mas-Coma S. Neglected human fascioliasis case in a visceral leishmaniasis endemic area, north-western Iran. Iranian J Publ Health. 2010;39:129-131.
12. Mohebali M, Hajjaran H, Hamzavi Y, Mobedi I, Arshi S, Zarei Z, Akhoundi B, Naeini KM, Avizeh R, Fakhar M. Epidemiological aspects of canine visceral leishmaniosis in the Islamic Republic of Iran. Vet Parasitol. 2005;129:243-251.
13. Ashrafi K, Saadat F, Rahmati B. Evaluating the level of endemicity of human fascioliasis in rural communities of Bandar-e-anzali district; an important step for implementation of disease control programs. *First International and 8th National Congress of Parasitology and Parasitic Diseases in Iran*. 2012:121
14. WHO. The" neglected" neglected worms. Action against worms. December 2007, issue 10. 2010
15. Mas-Coma S. Epidemiology of fascioliasis in human endemic areas. J Helminthol. 2005;-79:207-216.
16. Fuentes M, Valero M, Bargues M, Esteban J, Angles R, Mas-Coma S. Analysis of climatic data and forecast indices for human fascioliasis at very high altitude. Ann Trop Med Parasitol. 1999;93:835-850.
17. Moghaddam AS, Massoud J, Mahmoodi M, Mahvi AH, Periago MV, Artigas P, Fuentes MV, Bargues MD, Mas-Coma S. Human and animal fascioliasis in Mazandaran Province, northern Iran. Parasitol Res. 2004;94:61-69.
18. Lotfy WM, Hillyer GV. Fasciola species in Egypt. Exp Pathol Parasitol. 2003;6:9-22.
19. Soliman MF. Epidemiological review of human and animal fascioliasis in Egypt. The Journal of Infection in Developing Countries. 2008;2:182-189.
20. Zait H, Hamrioui B. [new cases of human fascioliasis in Algeria]. Medecine Tropicale: revue du Corps de sante colonial. 2005;65:395.
21. Qureshi AW, Tanveer A, WAHEED S, QURESHI A, GILL TJ, ALI SA. Epidemiology of human fasciolosis in rural areas of Lahore, Pakistan. Punjab Univ. J. Zool. 2005;20:159-168.
22. Farag H. Intestinal parasitosis in the population of the Yemen Arab republic. Trop Geog Med. 1985;37:29.
23. Sarkari B, Ghobakhloo N, Moshfea A, Eilami O. Seroprevalence of human fasciolosis in a new-emerging focus of fasciolosis in Yasuj District, southwest of Iran. Iranian J Parasitol. 2012;7:15-20.
24. Abdi J, Naserifar R, Rostami Nejad M, Mansouri V. New features of fascioliasis in human and animal infections in Ilam Province, western Iran. Gastroenterology and Hepatology from Bed to Bench. 2013;6:152-155.