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Iranian Society of Parasitology  
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### Original Article

## Serological study of Human Fasciolosis in Patients Referring to the School of Public Health, Tehran University of Medical Sciences, Tehran, Iran during 2008-2014

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Received 15 May 2015

Accepted 18 Sep 2015

#### **Keywords:**

Fascioliasis,  
ELISA,  
Raw Vegetable,  
Iran

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#### **Abstract**

**Background:** Fascioliasis is a zoonotic disease of livestock and human caused by *Fasciola* species. Here in, the results of serological evaluation of fascioliasis in people referring to the School of Public Health, Tehran University of Medical Sciences during 2008-2014 are presented.

**Methods:** Demographic characterizations, symptoms and eosinophil rate were registered for every patient. Using somatic antigen of *Fasciola*, ELISA was performed and the results were analyzed. Data of questioners were analyzed as well.

**Results:** Among 206 applicants, 24.8% were seropositive for fascioliasis, included 21% female and 28.3% male. Mean range of age of patients was between 13 to 67 yr. The highest rate of seropositivity was found among 20-30 yr old patients. Most of the patients had hypereosinophilia. All patients had history of eating raw vegetables, or drinking unsafe water. Patients were referring from different provinces of Iran, including Gilan, Mazandaran, Tehran, Ardabil, Khuzestan, Lorestan, North Khorasan, Kermanshah, Azerbaijan, Fars, Kordestan, Hamedan and Markazi.

**Conclusion:** During recent years, variety of provinces in Iran, where patients with fascioliasis are referred, has been increased. Patients coming from Gilan and Mazandaran provinces were referred early after the onset of their symptoms. Most probably, physicians in Gilan and Mazandaran are more alert on fascioliasis than other provinces. Previous wrong diagnosis was more common among patients referring from other provinces than Gilan and Mazandaran provinces.

## Introduction

Fascioliasis, caused by *Fasciola* spp., is a zoonotic helminthic diseases included among the neglected tropical diseases by WHO (1). Iran is amongst the MENA countries where human fasciolosis is prevalent mostly in northern areas and so far, two epidemics of high incidence rate have been reported (1-3). The diagnosis of human fasciolosis in Iran mostly is conducted using ELISA, which has showed acceptable sensitivity and specificity (4). Although parasitological diagnosis of the disease is considered the highest rate of specificity but the rate of sensitivity is low and some obstacles including transit infection, released parasite eggs, needing a specific method etc. are regarded as negative points on this method (1, 5). Although parasitological diagnostic technique has the highest rate of sensitivity, some obstacles including scanty of released parasite ova, spurious or transient infection, acute and obstructive infections decrease the rate of sensitivity of these methods. To overcome this problem an ELISA test, using somatic antigen, was established and set-up in the Laboratory of Serological Diagnosis of Helminthic Diseases of Department of Medical Parasitology and Mycology, School of Public Health, Tehran University of Medical Sciences, Iran. This test was used as a free of charge and authentic diagnostic method to ease the diagnosis of human fasciolosis throughout Iran. Many patients are referred to this laboratory, which considered somehow the main center for diagnosis of the disease.

The present report is to sketch a brief report of patients referred to this center during different times to assist policy makers on monitoring the present situation of fasciolosis in Iran.

## Materials and Methods

### Patients

Patients referred from different parts of the country to this diagnostic center were included in present study. They either were come by

themselves or were referred by physicians from private clinics, health care units or hospitals due to the presence of clinical symptoms or eosinophilic peripheral blood. In this retrospective descriptive study, the medical records of patients attended these centers during 2008 to 2014 were reviewed. Informed consent was taken from each patient. A questionnaire including information on location, diet, vegetable consumption, travelling to northern Iran, clinical symptoms etc. was filled out for each patient. Young patients or cases that were unable to fill out the form were aided by guardians. Blood was taken from all patients and sera were stored in refrigerator until examination. All sera were tested using Indirect ELISA established already.

Patients registered positive for fascioliasis were identified; among them, any one whose clinical characteristics and demographic data were completely available was included in this analytical study. There were also some fascioliasis infected cases with incomplete information; most of them related to patients who could not refer to the centers by themselves due to long distance, elderliness, hospitalization and so on. Therefore, only their serum samples were sent to the laboratories for examination. These patients were not enrolled in this study.

### Antigen preparation

For antigen preparation, *Fasciola* infected livers were prepared from Tehran slaughterhouses and transferred to the laboratory. After isolation of parasites from liver, and washing for 5 times with 0.045 mM PBS, they were homogenized with electrical homogenizer. After homogenization, sonication was carried out and centrifuged in 10000 g at 4 °C for 15 min. The supernatant was kept in refrigerator for later usage (4).

### ELISA test

ELISA test was conducted based on previously reported study with some modifications

(4). Briefly, 100 microliters of somatic antigen (10 mg/ml) was added to wells of plate sand incubated at 37 °C for 2 h then stay overnight at 4 °C. Plates were washed 3 times with PBS/Tween 20 then 200 microliters of gelatin 1% was added to wells of platesand incubated at 37 °C for two hours. Wells of plates were washed with PBS/Tween 20 for three times. One hundred microliters of a serum samples (diluted1:500) was added to wells coated with antigen and incubated at 37 °C for 30 min. Plates were washed 5 times with PBS/Tween 20. Peroxidase-conjugated goat anti-human IgG (diluted 1:10000) was added to wells and incubated at 37 °C for 30 min. After final washing step with PBS/Tween 20, 100 microliters of OPD was added to wells and reaction

was stopped with adding 50 microliters of stopper solution (12.5% H2SO4). OD was measured at492 nm with ELISA reader.

**Statistical analysis**

Statistical analysis was done using SPSS version16 (Chicago, IL, USA). Chi square test was used for analyzing of data. Cut-off was calculated as mean± 3 SD.

**Results**

Table 1 shows distribution of the patients based on the sex, age groups and percent of seropositivity in different age groups. Cut-off was calculated as 0.36.

**Table 1:** Age and sex distribution of fascioliasis in patient referring to the School of Public Health, TUMS during 2008-2014

Sex/ Age groups (yr)	No. of cases examined		No. of positive cases		Percent of infection		Total percent of infection
	Female	Male	Female	Male	Female	Male	
11-20	14	13	3	1	21.4	7.7	14.8
21-30	18	25	6	11	33.3	44.0	39.5
31-40	25	12	3	5	12	41.7	18.9
41-50	12	13	4	4	33.3	30.8	32.0
51-60	13	20	4	5	30.8	25.0	27.3
61-67	14	16	1	2	7.1	12.5	10.0
Unknown	4	7	0	2	0	28.6	18.2
Total	100	106	21	30	21	28.3	24.8

Overall, the rate of seropositivity was 24.8% for fascioliasis. Of 206 examined cases, 51.4% were male and 48.5% were female. The age of the patients varied from 13- 67 yr. The highest positiverate was in age group of 21-30 yr old. In 51 patients, two patients were

asymptomatic, 35 patients (68.6%) reported liver pain, and 12 patients (23.5%) complained of cutaneous symptoms including itching and rash. Pulmonary symptoms were recorded in 13 patients (25.5%) (Table 2).

**Table 2:** Distribution of patients infected with fascioliasis according to the sex and eosinophilia

Clinical manifestations	Liver symptoms	Pulmonary symptoms	Cutaneous symptoms	Fever
Sex	n (%)	n (%)	n (%)	n (%)
Female (n=21)	19(90.5)	6(28.6)	6(28.6)	14 (66.7)
Male (n=30)	16(53.3)	7(23.3)	6(20.0)	2(6.7)
Total (51)	35 (68.6)	13 (25.5)	12 (23.5)	26 (50.9)

Clinical symptoms were not significant between male and female patients. Determination of relative eosinophil count could be possible for patients, all of them showed more or less peripheral eosinophilia, ranging from 6% to 66%. 32.3% of patients had eosinophilia >30% and 67.6% <30%.

Patients were referred from different provinces of Iran. The highest infection rate was

seen in patients from Gilan & Mazandaran Provinces, northern parts of the country (37.3%). Nine patients (17.6%) were from Tehran, but all of them had the history of traveling to north of Iran. The distribution of these people according to their place of living is illustrated in (Table 3). All patients encompassed history of eating raw vegetables, or drinking unsafe water.

**Table 3:** Distribution of patients infected with fascioliasis according to their province of residence

Province	No. of case examined	No. of positive cases	Rate of infection (%)
Ardabil	7	4	7.9
Azerbaijan	1	1	2.0
Esfahan	3	2	3.9
Fars	1	1	2.0
Gilan & Mazandaran	65	19	37.3
Hamadan	2	1	2.0
Kermanshah	2	2	3.9
Khorasan	2	2	3.9
Khuzestan	3	2	3.9
Kordestan	3	1	2.0
Lorestan	7	3	5.9
Markazi	4	1	2.0
Tehran	77	9	17.6
Unknown	29	3	5.9
Total	206	51	100

As for polyparasitoses, 24.8% of patients were infected with fasciolosis only, 0.5% with fasciolosis and hydatidosis, simultaneously and 0.1% with fasciolosis and strongyloidiasis.

## Discussion

From 2008 to 2014, altogether 206 patients suspected for fascioliasis were referred to our center. Overall, 24.8% were seropositive using ELISA test. Clinical manifestations and paraclinical tests boosted the accuracy rate of diagnosis. Although the gold standards for diagnosis of fasciolosis is stool exam but documented issues attest a low sensitivity (6, 7), however many studies conducted so far approve the outstanding role of ELISA test on diagnosing fasciolosis (4, 8-11).

The situation of human fasciolosis in Iran needs permanent monitoring due to especial

characteristics and Food habitats found there. Iran has experienced two large outbreaks of human fascioliasis in 1989 and 1999 affected more than 15000 infected cases (12-14). However, recent survey has revealed prevalence of 0.4% and 1.2% using coprological and serological methods, respectively, regarded the area as hypoendemic. (15). World Health Organization (WHO) considers Iran among six countries known to have serious Problem with fascioliasis (16). It is worth mentioning that some new reports have been put forwarded which demands more cautionary measures to monitor the disease in different regions (1, 17-19).

We noticed the highest infection rate in 20-30 yr old. 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 (15, 18). In

Iran, the numbers of infected cases were higher in older (> 20 year) age groups during non-epidemic situations. The pattern of age wise prevalence shows that adults are infected more than children in Iran (1, 2).

The present study shows that cases of human fascioliasis occur in different parts of the country and permanent monitoring should be done by health authorities and policy makers for preventing the occurrence of another outbreak especially in the northern part. Reporting of new foci of human fascioliasis from Yasuj (17), Kermanshah (18), Lorestan (Unpublished data), verifies this idea.

During recent years, the number of provinces from which human cases of fascioliasis are referred to our center has increased. Patients coming from Gilan and Mazandaran provinces were referred soon after the onset of their symptoms because local physicians are more alert on fascioliasis than their counterparts working in other provinces are. On the other hand, most initial wrong diagnosis were among those patients referred from provinces other than Gilan and Mazandaran

## Conclusion

Although combating any parasitological disease sounds difficult and sometimes impossible, but fortunately, previous experiences in Iran in terms of elimination the helminthic diseases attest that we are able to control human fascioliasis to an ideal extent and this target is accessible and achievable.

## Acknowledgements

The authors declare that there is no conflict of interests.

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