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Seroepidemiology of *Fasciola Hepatica* in Mersin province and surrounding towns and the role of family history of the Fascioliasis in the transmission of the parasite

Mersin ili ve ilçelerinde *Fasciola hepatica* seroepidemiyolojisi ve bulaşta aile öyküsünün önemi

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Background/aims: *Fascioliasis is an important zoonotic disease caused by Fasciola hepatica. This zoonosis may cause serious morbidity and a considerable financial burden. Knowledge about Fasciola hepatica and interest in this parasite have increased in Turkey recently. However, there have been few studies on the real prevalence of this condition in the country. Therefore, we aimed to determine the prevalence of fascioliasis and the role of family history of the condition in the transmission of the parasite in the province of Mersin.* ***Methods:*** *Taking account of their populations, 729 people without a family history of fascioliasis and 155 people with a family history of fascioliasis from the city of Mersin and randomly selected three towns were included into the study to obtain a sample that well represented the population of the province of Mersin. A questionnaire composed of items about consumption of green leafy vegetables, stock-breeding and clinical symptoms of the disease was used to collect data. Excretory/secretory (ES)-ELISA was used to detect IgG antibodies to Fasciola hepatica. People seropositive for Fasciola hepatica underwent abdominal ultrasonography, physical examination, biochemistry, and stool tests for the detection of Fasciola hepatica eggs.* ***Results:*** *A total of 0.79% of the participants were seropositive for Fasciola hepatica. One point ninety-three percent of the individuals with a family history of fascioliasis and 0.55% of the individuals without a family history of fascioliasis were seropositive for Fasciola hepatica. Out of 7 individuals found to be seropositive for Fasciola hepatica, 5 were female, 2 were male, and 4 had a family history of fascioliasis. Five and 4 patients, respectively, had a history of consuming green leafy vegetables and 4 had a history of stock-breeding. The clinical evaluation revealed that 4 patients had at least one sign of fascioliasis. Three patients had signs of fascioliasis on ultrasonography and 1 had Fasciola hepatica egg in stool examination. There was no significant difference in seropositivity for Fasciola hepatica between the individuals with and without a family history of fascioliasis (x2: 0.077, p>0.05).* ***Conclusions:*** *The prevalence of fascioliasis was hypoendemic in the province of Mersin. There were no significant differences in the Fasciola hepatica prevalence between the groups with and without family history of fascioliasis. However, studies with larger sample sizes may reveal a difference.*

Key words: Fasciola hepatica, seroepidemiology, excretory/secretory-ELISA.

Amaç: *Fasciola hepatica önemli sağlık sorunlarına ve ekonomik kayıplara neden olabilen ciddi bir zoonotik hastalıktır. Son yıllarda ülkemizde bu zoonoz'a yönelik ilgi ve bilgi birikiminin arttığını biliyoruz. Fakat yine de bu parazitin ülkemizdeki gerçek sıklığı konusunda maalesef yeterli çalışma ve bilgi yoktur. Bu amaçla ülkemizdeki fasciola bilgi birikimine önemli kaynak oluşturan Mersin İli ve İlçelerinde Fasciola hepatica sıklığını saptamak ve aile öyküsünün fasciola sıklığındaki etkisini değerlendirmek amacı ile bu çalışmayı planladık.* ***Yöntem:*** *Mersin ilinin tümünü örnekleyecek şekilde kura ile belirlenen 3 ilçe ve Mersin merkez olmak üzere nüfus oranları dikkate alınarak ailesinde fasciolosis öyküsü bulunmayan 729 ve fasciolosis öyküsü bulunan 155 kişi çalışmaya alındı. Çalışma grubundaki kişilere yeşillik yeme, hayvan besleme ve klinik semptomlara yönelik anket formu düzenlendi ve doldurtuldu. Fasciola hepatica IgG antikorları ES-ELISA yöntemi ile araştırıldı. Seropozitif olan hastalar tekrar değerlendirilerek abdominal ultrasonografileri, fizik muayeneleri, biyokimyasal tetkikleri ve gaitada parazit yumurtası incelemesi yapıldı.* ***Bulgular:*** *Çalışma grubunda toplam seropozitiflik oranı %0.79 olup, ailesinde fasciolosis bulunan kişiler arasında seropozitivite %1.93, bulunmayanlarda ise %0.55 olarak saptanmıştır. Seropozitiflik saptanan 7 hastanın 5'i kadın 2'si erkek olup 4'ünde fasciolosis aile öyküsü bulunmaktadır. Hastaların 5'inde yeşillik yeme, 4'ünde hayvan besleme öyküsüne, klinik değerlendirmede ise 4 hastada en az bir fasciolosis düşündürebilecek bulguya rastlanmıştır. Seropozitif 7 hastanın ultrasonografik incelenmesinde 3 hastada fasciolosis ile uyumlu bulgular elde edilirken, dışkı incelemesi sonucunda 1 hastada Fasciola hepatica yumurtası görülmüştür. Aile öyküsü bulunan ve bulunmayan gruplar arasındaki farkın istatistiksel olarak anlamlı olmadığı tespit edilmiştir (p>0.05).* ***Sonuç:*** *Çalışmamızda Fasciola hepatica sıklığı Mersin ili genelinde %0,8 olup hipoendemik bir bölge olduğu ortaya çıkmıştır. Aile öyküsü olanlar ile olmayanlar arasında seropozitiflik açısından istatistikˆ fark bulunmamış olsa da yaklaşık olarak 4 kat fark bulunmuştur. Olgu sayısının artması durumunda anlamlı farklılık olabileceğini düşünüyoruz. Metaserkaryaların üzerinde bulunduğu su teresi gibi bitkilerin yenmesi ve enfekte suların içilmesi ile hastalık bulaşabildiğinden Fasciola hepatica tespit edilen kişi ile ortak kaynaktan beslenen kişilerde de hastalığın olabileceği düşünülerek ailelerinin de taranması ve serolojik tanının, dışkıda yumurta aranması ve radyolojik yöntemlerle desteklenmesi sonucuna varılmıştır.*

Anahtar kelimeler: Fasciola hepatica, seroepidemiyoloji, fasciolosis, ELISA

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INTRODUCTION

Fascioliasis, referred to as "big liver butterfly" in Turkey, is frequently encountered in ruminants (1, 2). Since it rarely appears in human beings, fascioliasis has been disregarded until recently. However, with the recent introduction of advanced diagnostic methods, the incidence of fascioliasis has turned out to be higher than expected in human beings. In fact, clinical features of the disease have been better described (3-5). People and animals can contract the disease when they eat plants such as watercress, which contain metacercaria, and when they drink water contaminated by metacercaria or use kitchenware contaminated by metacercaria (3, 6, 7). Lymnaeidae molluscs are the intermediary hosts of *Fasciola hepatica* and the most important intermediary host of *Fasciola hepatica* is *Lymnaea truncatula,* which is encountered in all parts of Turkey (8, 9). Consumption of large amounts of freshwater plants, which varies from region to region, suggests that the incidence of the disease can be higher than expected (3, 6).

It has been recently reported that 2.4 million people have *Fasciola hepatica* and 180 million people are the risk of infection with *Fasciola hepatica*. In Turkey, the diagnosis of fascioliasis is based on extraction of adult parasites during surgeries or endoscopies for obstructive jaundice, chronic cholecystitis, cholangitis, cholelithiasis, hepatitis, or gallbladder tumor (10-17).

The aim of this study was to determine seroprevalence of fascioliasis in the province of Mersin, and to reveal the relation between infestation and family history of fascioliasis.

MATERIALS AND METHODS

Study Population

Two study groups were formed: one including 729 individuals without a family history of fascioliasis (Group 1) and the other including 155 individuals with a family history of fascioliasis (Group 2). Participants of Group 1 were from the city of Mersin and its randomly selected towns Tarsus, Bozyazı and Mut. Group 2 included 8 patients with fascioliasis presenting to the Gastroenterology Outpatient Clinic of Mersin University Medical School Research and Training Hospital, their families and relatives. The diagnosis of fascioliasis in Group 2 patients was based on clinical, cholangiographic and ultrasonographic findings and confirmed by enzyme linked immunosorbent assay (ELISA) in the Serology Laboratory of the Parasitology Department of Ege University Medical School. All participants completed a questionnaire containing items regarding history of stock-breeding and consumption of green leafy vegetables, abdominal pain, nausea, vomiting, fever, redness, itching, and weight loss.

Laboratory Investigations

Venous blood specimens of 7-8 ml were obtained from all participants and centrifuged at 3000xg for 5 min, and the obtained serum specimens were kept in Eppendorf tubes at -20°C. ELISA was used to detect IgG antibodies to *Fasciola hepatica* in the serum specimens. Individuals found to have *Fasciola hepatica* were contacted and invited to give blood samples for complete blood count, eosinophil count, and erythrocyte sedimentation rate, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and gamma glutamyl transpeptidase (GGT) measurements. Three stool specimens were collected on alternate days from the individuals found to be seropositive for *Fasciola hepatica* on ELISA and the specimens were homogenized in 10% formalin. They were then examined for detection of *Fasciola hepatica* egg with formalin ethyl acetate method. The individuals seropositive for *Fasciola hepatica* also underwent ultrasonography.

ELISA Procedures

Obtaining *Fasciola hepatica*

Living adult *Fasciola hepatica* were removed from the bile ducts of the naturally infected cattle immediately after slaughtering in a slaughterhouse and rinsed with sterile NaCl 9-10 times to remove blood and bile remains.

Obtaining Excretory/Secretory (ES) Antigens

First, phenyl methyl sulfonyl fluoride (PMSF) (Sigma) was added into phosphate buffered saline solution (PBS), and sterile PBS-PMSF solution containing 2 mM PMSF was prepared. Next, for each living adult *Fasciola hepatica,* 1 ml PBS-PMSF was put into sterile flasks and incubated at 37oC for 3 h. Then, adult *Fasciola hepatica* were removed and the obtained fluid was centrifuged at 4500 cycles/min and at +4oC for 1 h. Finally, the supernatant was filtered through a 0.2 μm filter (Minisart) and the filtered fluid was transferred into 1 ml Eppendorf tubes, each having 128 mg/dl protein, and kept at -60°C.

ELISA

ELISA involved two stages. In the first stage, for each ELISA plate, 11 ml carbonate-bicarbonate buffer solution was added to 200 µl-*Fasciola hepatica* ES antigens and the obtained fluid was put into 96-well ELISA plates, each well containing 100 µl of the obtained solution. In the second stage, antigen-coated ELISA plates were incubated at 4°C overnight and in the morning their contents were removed. Serum specimens were diluted with PBS-CB at the rate of 1/100. Two wells were used for each serum specimen. A substrate containing recombinant anti-human IgG conjugate (ZYMED) labelled with peroxidase enzyme diluted with PBS-CB at 7/10000 and 10 ml TMB-citrate buffer combined with 1 ml TMB was used. Optic densities were determined with ELISA plate readers at 450 nm wavelength.

Statistical Analyses

Chi-square test was used to determine the significance of the seroprevalence rates in the groups with and without family history of fascioliasis.

RESULTS

Group 1

Group 1 consisted of 249 (34.2%) males and 480 (65.8%) females. Their ages ranged between 15-69 years. The mean age of the males and females was 39.7 years and 39.9 years, respectively. Six hundred and forty-four people (88.3%) had a family history of consuming green leafy vegetables and 111 (15.2%) had a family history of raising livestock (goats, sheep, cattle, cows, and bulls).

Group 2

Group 2 included 71 (45.8%) males and 84 (54.2%) females. The participants were aged 15-69 years with a mean age of 37.2 years. One hundred and forty-six (94.2%) had a history of consuming green leafy vegetables and 43 (27.7%) had a history of raising livestock (cows, sheep, bullocks, donkeys, goats, and calves).

Blood specimens were collected again from individuals found to be seropositive in order to perform other analyses. A total of 7 people (0.8%), of whom 4 (0.6%) were in Group 1 and 3 (1.9%) were in Group 2, were seropositive for fascioliasis. Out of 7 people, 5 (71.4%) were female with a mean age of 28.6 years and 2 (28.6%) were male with a mean age of 53 years. Data about the 7 people seropositive for fascioliasis obtained through the questionnaire are presented in Table 1. Laboratory results of 7 patients seropositive for fascioliasis are shown in Table 2.

Ultrasonographic findings and stool examination results of 7 patients with IgG positivity are shown in Table 3.

Ultrasonographic view and the *Fasciola hepatica* egg detected in the stool examination of patient no. 5 are shown in Figures 1 and 2, respectively.

There were no significant differences in the *Fasciola hepatica* seroprevalence between the groups with and without family history of fascioliasis (x^2=0.077, p>0.05).

Table 1. Data about the 7 individuals seropositive for fascioliasis as obtained through the questionnaire

	Patients	Age	Gender	History of eating green vegetables	History of raising livestock
	1	27	F	Yes	Yes
Group 1	2	37	F	No	No
	3	39	F	No	No
	4	42	M	Yes	Yes
	5	22	F	Yes	Yes
Group 2	6	64	M	Yes	Yes
	7	18	F	Yes	No

Table 2. Laboratory results of 7 patients seropositive for fascioliasis

Patient	Complaints	WBC	Hb	Eo (%)	ESR	AST	ALT	ALP	GGT
1	None	6.15	14.4	7.2	33	49	63	78	22
2	None	5.96	12.5	5.7	2	19	16	47	19.8
3	Redness	7.36	11.3	8.6	37	17	19	76	6.2
4	None	6.44	12.1	4.2	39	41	46	95	18.6
5	Abdominal pain, itching and redness	5.38	11.9	4.5	26	19	11	78	9.7
6	Abdominal pain and itching	5.36	12.1	4.2	28	29	17	78	33
7	Abdominal pain, redness and weight loss	5.18	10.4	1.4	19	33	25	56	10.9

WBC: White blood cells. Hb: Hemoglobin. Eo: Eosinophil. ESR: Erythrocyte sedimentation rate. AST: Aspartate aminotransferase. ALT: Alanine aminotransferase. ALP: Alkaline phosphatase. GGT: Gamma glutamyl transpeptidase.

Table 3. Ultrasonographic findings and stool examination results of 7 patients with IgG positivity

Patient	Abdominal Ultrasonography	Eggs of *fasciola hepatica*
1	Gallbladder polyp	No
2	Normal	No
3	Normal	No
4	Normal	No
5	Common bile duct larger than normal (10 mm) and nearly full with formations with an echoic structure similar to the liver parenchyma	Yes
6	Structures in the gallbladder with echogenicity suggestive of Fasciola hepatica	No
7	Enlargement of the common bile duct (8 mm)	No

DISCUSSION

Fasciola hepatica is a zoonosis that resides in the bile ducts of humans and animals such as sheep, goat and cattle and which causes fascioliasis. This infection is sporadic in Turkey and is recognized during surgical or endoscopic procedures. Eosinophilia, unknown fever, atypical abdominal pain, family history of fascioliasis, history of consumption of green vegetables, biliary colic or cholangitis, and focal intrahepatic lesions indicate fascioliasis (3, 5, 18, 19).

There have been few studies on the epidemiology of *Fasciola hepatica*. The first study on the seroprevalence of fascioliasis in Turkey, carried out on people presenting to the first-line health care centers in the province of Antalya (20), revealed that 3.0% of the study population were seropositive for fascioliasis. This study showed that Antalya was mesoendemic for fascioliasis according to the classification by Mas-Coma et al (6). Another study was performed in the province of Elezığ and revealed that the rate of seropositivity was 2.8% (21). Demirci (9) considered high rates of eosinophilia as the main indicator of fascioliasis and reported that 6.1% of 756 people with eosinophilia and 0.9% of 320 people without eosinophilia were seropositive for fascioliasis. The high incidence of fascioliasis in the provinces of Isparta and Antalya was explained by the fact that people residing in these provinces raise herds of sheep or goat, are dependent on agriculture for their income, and consume large amounts of plants such as watercress, since there are many natural water sources, lakes and water canals and plants such as watercress grow in areas where water is plentiful (9, 20). In the present study, history of fascioliasis was considered as the main indication of fascioliasis. One point ninety-three percent of individuals with history of fascioliasis and 0.55% of individuals without family history of fascioliasis were seropositive for *Fasciola hepatica.* Although the incidence of fascioliasis has been reported to be nearly the same in both genders, in this study, out of 7 patients with fascioliasis, 5 (71.4%) were female and 2 (28.6%) were male. This can be attributed to the fact that 63.8% of the study sample was female.

It has been reported that infected herbivorous mammalians play an important role in the transmission of fascioliasis, but that the prevalence of the disease in humans is not high in the regions where the prevalence of the disease among animals is high. The source of infection among humans is water plants contaminated with metacercaria. Apart from eating water plants such as Nasturtium officinale (watercress), drinking water contaminated with metacercaria and using kitchenware contaminated with metacercaria may also play a role in the disease transmission (3, 6, 7). In the present study, high rates of individuals in both groups were found to consume watercress. In fact, out of patients seropositive for *Fasciola hepatica,* 71.4% had a history of consuming green vegetables and 51.1% had a history of raising livestock.

Fascioliasis may cause a wide variety of clinical signs ranging from asymptomatic infection to severe liver cirrhosis. In our study, we determined some nonspecific symptoms such as abdominal pain, redness, itching, and weight loss. Three out of 7 patients had no signs suggestive of fascioliasis.

Although it is known that investigation of *Fasciola hepatica* eggs in the stool is a standard method for the diagnosis of fascioliasis, the parasites do not pass eggs in the acute stage of the disease when it has not yet become an adult, although the symptoms of the disease are the most severe. In addition, parasite eggs may not be detected when the parasite lays at intervals in cases of chronic fascioliasis and when the parasite has an ectopic location (3, 7, 22, 23). In the seroepidemiological study in the province of Antalya, stool was examined in 16 patients in whom serum IgG antibodies to *Fasciola hepatica* were found using ES-ELISA method, but only 4 (25%) had eggs of *Fasciola he-*

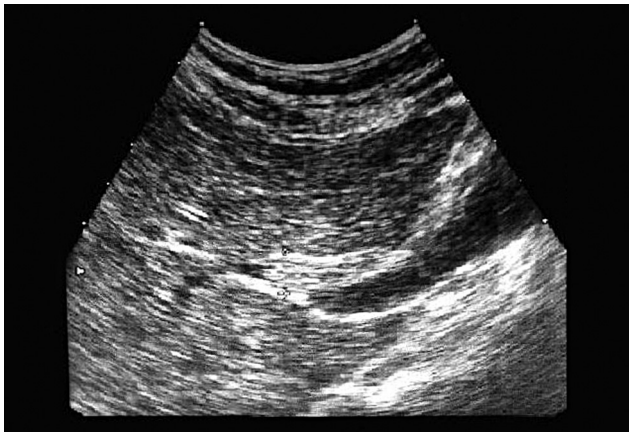

Figure 1. Ultrasonographic view in patient no. 5 shows the common bile duct as larger than normal (10 mm) and nearly full with formations with an echoic structure similar to the liver parenchyma.

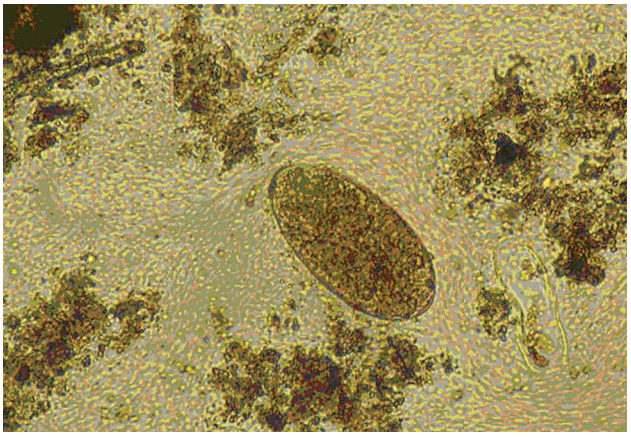

Figure 2. The Fasciola hepatica egg detected in the stool examination of patient no. 5.

*patica (*19). Similarly, we found that only one patient (14.3%) with IgG positivity had eggs of *Fasciola hepatica* in their stool.

Eosinophilia is a sign of fascioliasis. Turhan (20) found that 2 (11.1%) out of 18 patients seropositive for *Fasciola hepatica* on ES-ELISA method had eosinophilia. We also observed that 43% of seropositive patients had eosinophilia.

At present, serological tests are known to be the most valuable diagnostic tests and can be used in all stages of the disease, including the acute stage when the eggs are not yet excreted in the stool, and in the evaluation of the treatment outcome (3, 23).

Adult *Fasciola hepatica* produce some substances during their life in the hosts, which vary with hosts and are called ES liquids or excreta. It has been reported that ELISA involving ES antigens is more sensitive in the diagnosis of *Fasciola hepatica* than other serological tests. ES-ELISA has the advantages of being inexpensive and easy to perform and it allows examination of many serum specimens at a time (24, 25). Taylan Özkan and Kuman (26) reported that the sensitivity and specificity of ES-ELISA were 100% and 95.3%, respectively, in the diagnosis of fascioliasis. Şakru (27), in his study on 15 cases in whom *Fasciola hepatica* was detected and 22 cases in whom the parasite eggs had not been detected despite repeated stool examinations, noted that the sensitivity and specificity of ES-ELISA were 100% and 97%, respectively. In the present study, although *Fasciola hepatica* eggs were detected in only one case, ES-ELISA detected IgG positivity in 7 cases.

Out of 7 patients found to have IgG antibodies, 3 had normal ultrasonographic findings. One had dilatation of the common bile duct; only 1 had dilatation of the common bile duct filled with formations with the same echogenicity as the liver tissue and 1 had echogenicity in the gallbladder. These latter two findings are highly suggestive of *Fasciola hepatica* (5). The fourth patient (no. 1) with abnormal ultrasonography had a gallbladder polyp.

In conclusion, our study showed that the seroprevalence rate indicated hypo-endemicity in Mersin and that family history was not significant in *Fasciola hepatica* infestation. However, we believe that further studies are needed to elucidate the significance of this issue.

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