

Investigation of *Fasciola hepatica* Seropositivity in Forensic Autopsies in the Çukurova Region

Çukurova Bölgesindeki Adli Otopsilerde *Fasciola hepatica* Seropozitifliğinin Araştırılması

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ABSTRACT Objective: *Fasciola hepatica* is a parasite that affects ruminant animals and is rarely seen in humans. The agent may cause significant health problems and economic loss as a zoonotic disease. Freshwater plants play an important role for infecting humans with this parasite. Eating watercress, drinking water that contains metacercaria, using contaminated kitchen tools, or consuming green vegetables may cause infestation. Green vegetables and fruits, especially watercress, are grown specifically in the Çukurova region and this puts the population living in this region at risk of developing fasciolosis. For the reason of absence of the seroprevalence studies in autopsies and to determine the prevalence of this region, the aim of this study, is to investigate *F. hepatica* antibody by the method of IHA blood samples which were taken from autopsy cases in the Çukurova Region. **Material and Methods:** This study was conducted in Adana Forensic Medicine Department, a center where all forensic autopsies from the cities of Adana, Mersin, Kahramanmaraş, Hatay and Osmaniye were performed. There were 94 subjects between the ages of 2-87 years (mean:42.55±SD22.09) and their serum samples were collected for assessment of *F. hepatica* antibodies via the IHA method. Moreover, we examined the hepatobiliary system for adult parasites. **Results:** IHA results demonstrated that 13 (13.8%) of the subjects were seropositive for *F. hepatica*. During the autopsy, however, mature *F. hepatica* was not observed in hepatobiliary systems of the subjects. Seropositivity rates in Adana, Mersin, Hatay and Osmaniye were 19.4%, 7.1%, 12.5% and 22.2%, respectively. **Conclusion:** To our knowledge, this is the first study evaluating *F. hepatica* seropositivity in forensic autopsies. Seeing the results, we suggest that more comprehensive epidemiologic research is needed to study this infestation, especially in endemic areas.

Key Words: *Fasciola hepatica*; autopsy; forensic medicine

ÖZET Amaç: Geviş getiren hayvanların paraziti olan *Fasciola hepatica* insanlarda nadir görülen, önemli sağlık sorunlarına ve ekonomik kayıplara neden olabilen zoonotik bir hastalıktır. Parazitin insanlara bulaşmasında tatlı su bitkileri önemli rol oynamaktadır. Hastalık metaserkaryanın bulunduğu su teresinin yenilmesi, suların içilmesi veya bunlarla kontamine olmuş mutfak aletlerin kullanılması ve yeşil sebzelerin tüketilmesiyle bulaşabilmektedir. Çukurova Bölgesinde özellikle su teresi gibi yeşil sebze ve meyveler bol miktarda tüketilmektedir, bu nedenle birçok insan fasciolosis bakımından büyük bir risk altındadır. Otopsilerde seroprevalans çalışmamaların olmaması ve bu bölgedeki yaygınlığın belirlenmesi amacıyla bu çalışmada, Çukurova Bölgesinde adli otopsi vakalarından alınan kan örneklerinde, IHA yöntemi ile anti-*F. hepatica* antikorlarının araştırılması amaçlanmıştır. **Gereç ve Yöntemler:** Araştırma Adana, Mersin, Kahramanmaraş, Hatay ve Osmaniye illerini kapsayan, Çukurova Bölgesinde adli otopsilerin yapıldığı ana merkez olan Adana Adli Tıp Grup Başkanlığında gerçekleştirilmiştir. Anti-*F. hepatica* antikorları, otopsi yapılan yaşları 2 ile 87 (ortalama:42,55±SD:22,09) arasında değişen toplam 94 kişinin serum örneklerinde IHA yöntemi ile araştırılmıştır. Ayrıca otopsi sırasında karaciğer ve safra yollarında parazitin erişkini araştırılmıştır. **Bulgular:** Çalışmamızda *Fasciola hepatica* antikorları yönünden incelenen otopsilerin 13 (%13,8)'ünde seropozitiflik saptanmıştır. Ancak otopsileri yapılan kişilerin hiçbirinde karaciğer ve safra yollarında yapılan incelemede *F. hepatica*'nın erişkinine rastlanmamıştır. Seropozitiflik oranları; Adana'da %19,4, Mersin'de %7,1, Hatay'da %12,5, Osmaniye'de %22,2 olarak bulunmuştur. **Sonuç:** Çukurova Bölgesinde ve Türkiye de adli vakalarda *Fasciola hepatica* seropozitifliğinin araştırıldığı bu ilk çalışma sonuçlarına göre, enfeksiyonun özellikle endemik bölgelerde daha geniş kapsamlı epidemiyolojik araştırmalarının yapılması gerektiğini düşünmekteyiz.

Anahtar Kelimeler: *Fasciola hepatica*; otopsi; adli tıp

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F*asciola hepatica* is a parasite that affects ruminant animals and is rarely seen in humans. The agent may cause significant health problems and economic loss as a zoonotic disease. Freshwater plants play an important role in the infestation of humans by this parasite. Eating watercress, drinking water that contains metacercaria, using contaminated kitchen tools, or consuming green vegetables may cause infestation.

Fasciola hepatica prefers to be located in the hepatobiliary system, but it often remains asymptomatic. The severity of hepatobiliary injury after infestation depends on the amount of parasites ingested. Biliary epithelial hyperplasia and fibrosis in the portal or biliary tract is often seen. In addition, hepatic abscesses may occur after hepatic invasion. Chronic infestation may present with malaise, gastrointestinal complaints, fever, right upper quadrant pain, hepatomegaly and occasionally jaundice. Mortality is rare and is generally due to biliary obstruction related to infestation.¹ Ectopic localization may occur as well.²

Observing the eggs of the parasite in a subjects' stool is usually sufficient for diagnosis.^{1,3,4} However, to observe the eggs of the parasite in the stool, *F. hepatica* taken from the last host must complete its migration to the liver, mature in the biliary tract and lay its eggs; and this process can last approximately 3-4 months. In chronic phase, the parasite lays its eggs intermittently and not many parasite eggs remain in the stool, or no eggs remain in the acute and/or ectopic cases; hence, there is a possibility of developing a pseudo-parasite, therefore stool examination alone is not sufficient for diagnosis.^{3,5} Serologic tests are therefore the standard methods for the Fasciolosis diagnosis.

Fasciola hepatica infestation is usually seen in rural areas or in cities with close relationships with rural areas. Poor personal hygiene, water sanitation issues and consuming raw vegetables can increase the likelihood of infestation.⁶

The Cukurova region has an increased risk of Fasciolosis based on the consumption of large amounts of watercress and fruits. For the reason of absence of the seroprevalence studies in autopsies

and to determine the prevalence of this region, the aim of this study is to investigate *F. hepatica* antibody by the method of IHA blood samples which were taken from autopsy cases in the Çukurova Region.

MATERIAL AND METHODS

This study was conducted in Adana Forensic Medicine Department, which is a center where all forensic autopsies from the cities of Adana, Mersin, Kahramanmaraş, Hatay and Osmaniye were performed. The study was approved by the Council of Forensic Medicine Medical Ethics Committee.

Autopsies due to suicide, accident or murder were performed on 94 subjects. During autopsy, 5 ml of blood was taken intracardially or intrafemorally and the samples were then centrifuged at 3000 xg. Moreover, we examined the hepatobiliary system for adult parasites. IHA was performed to assess anti-*F. hepatica* antibodies in the serum samples. Commercially available kits (Distomiasis Fumouze, Fumouze Laboratories, France) were used in accordance with the recommended procedures.

RESULTS

Evaluation was performed on 94 subjects for anti-*Fasciola hepatica* antibodies. There were 70 males (74.4%) between the ages of 2-84 years (mean:40.22±SD:20.50) and 24 females (25.6%) between the ages of 5-87 (mean:49.33±SD:25.46) years. Seropositivity was determined in 13 (13.8%) of the 94 subjects. However during the autopsies, the hepatobiliary tract examination failed to reveal the presence of any mature *F. hepatica* parasites. Table 1 shows the distribution of *F. hepatica* serological positivity at the autopsies in each city in the region of Çukurova.

Anti-*Fasciola* antibody seropositivity was 8.6% and 29.2% in males and females, respectively (Table 2).

F. hepatica seropositivity rates by gender in each city (Table 3).

TABLE 1: Distribution of *F. hepatica* serological positivity at the autopsies in each city in the region of Çukurova.

Group	Anti- <i>Fasciola hepatica</i> antibody (+)		Anti- <i>Fasciola hepatica</i> antibody (-)		Total	
	Number	%	Number	%	Number	%
Adana	6	19.4	25	80.6	31	100
Mersin	2	7.1	26	92.9	28	100
Kahramanmaraş	-	-	2	100	2	100
Hatay	3	12.5	21	87.5	24	100
Osmaniye	2	22.2	7	77.8	9	100
Total	13	13.8	81	86.2	94	100

TABLE 2: *F. hepatica* seropositivity rates by gender.

Gender	Anti- <i>Fasciola hepatica</i> antibody (+)		Anti- <i>Fasciola hepatica</i> antibody (-)		Total	
	Number	%	Number	%	Number	%
Male	6	8.6	64	91.4	70	100
Female	7	29.2	17	79.8	24	100
Total	13	13.8	81	86.2	94	100

TABLE 3: Distribution of genders of the subjects in each city.

Group	Male		Female		Total	
	Number	%	Number	%	Number	%
Adana	23	74.2	8	25.8	31	100
Mersin	22	78.6	6	21.4	28	100
Kahramanmaraş	2	100	-	-	2	100
Hatay	17	70.8	7	29.2	24	100
Osmaniye	6	66.7	3	33.3	9	100
Total	70	74.5	24	25.5	84	100

DISCUSSION

The frequency of infestation and importance of *Fasciola hepatica* has increased with the development of diagnostic methods and improved understanding of its pathogenesis, as well as identified novel clinical symptoms. A *Fasciola hepatica* diagnosis may be confused with a number of diseases and therefore may result in a delayed diagnosis, therefore serologic methods gain importance in both acute and chronic infestation. Fasciolosis is not only seen in individuals who live in rural areas, but also seen in individuals who live in urban areas in Turkey.⁵

There are no known studies that have investigated Fasciolosis prevalence in forensic autopsy subjects. The epidemiology of *F. hepatica* is influ-

enced by several factors including climate, soil features, lifestyles and diet of individuals, as well as the presence of the parasite's intermediate host, *Lymnaea truncatula*. In Cukurova region people consume watercress and it is endemic for this parasite, and the population has a substantial risk of infestation. Therefore we aimed to investigate the seropositivity of the parasite.

A high prevalence rate was observed in a number of countries including Australia, Iran, Egypt, Bolivia, Cuba, Ecuador, England, Peru, France and Portugal.⁷ The prevalence of Fasciolosis was 3% in Portugal, 7% in Egypt and 9% in Peru. The highest prevalence was found in Bolivia, where stool examination revealed a prevalence of 72%, and serologic tests were positive in 100%.^{8,9} Lopez et al.

investigated the prevalence of Fasciolosis in school age children in Peru and they reported that 10.3% had positive stool microscopy.¹⁰

There have been several epidemiologic studies on Fasciolosis in Turkey. In the Bagtepe village of Kozan/Adana, IHA was used and performed with a 1/320 dilution; seropositivity was seen in 3.78% of the population, whereas seropositivity was 3.01% in ten villages in Antalya and the Lakes region.^{5,11} In three counties centered around Mersin, ES-ELISA was performed and it was found that the prevalence of seropositivity against *F. hepatica* IgG antibody was 0.79%.¹² Using ELISA and IHA, the population in the Kayseri-Karpuzsekisi basin had a 3.48% seropositivity rate.¹³ A study in Elazığ reported that the prevalence of IgG seropositivity was 2.78%, whereas another study found that of foreign students in Kayseri, 3.5% were seropositive.^{5,14} In our study, seropositivity rates in the cities of Adana, Mersin, Hatay and Osmaniye were 19.4%, 7.1%, 12.5% and 22.2%, respectively.

Common livestock activities, abundant natural springs and irrigation ditches as well as a large consumption of watercress can cause Fasciolosis epidemics and it can become a major health concern.¹⁵ Therefore, the roles of public health specialists and primary health care providers are crucial in these areas.

To our knowledge, this is the first study evaluating *F. hepatica* seropositivity in forensic autopsies. Given the results, we suggest that more comprehensive epidemiologic research is needed to study this infestation, especially in endemic areas.

A larger sample size of autopsies in forensic medicine is needed for future studies. The ratio found in this study was 13%, which is high. We think that increasing the number of samples and utilizing ELISA and Western blotting techniques, more extensive seroprevalence studies need to be done.

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