

Increased anti-Echinococcus granulosus antibody positivity in Fasciola hepatica infection

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Background/aims: Parasitic helminths express some antigen, which often accounts for serological cross-reactions. The aim of this study was to determine the prevalence of anti- *Echinococcus granulosus* antibody in patients with *Fasciola hepatica* infection using indirect immunofluorescence assay. **Materials and Methods:** The study population consisted of the following groups: *Fasciola hepatica* group (n=22), hydatid disease group (n=22) and healthy control group (n=24). Indirect immunofluorescence assay for *Echinococcus granulosus* was performed in all groups. **Results:** Indirect immunofluorescence assay was positive in all patients with hydatid disease, in 13 of 22 (59%) patients with fascioliasis and in 2 of 24 (8%) healthy subjects. The positivity rate of indirect immunofluorescence assay was significantly higher in the hydatid disease group compared to the fascioliasis group (p<0.001) and compared to the control group (p<0.001), and it was significantly higher in the fascioliasis group compared to the control group (p=0.001). Antibody titer was 1/100 in 7 patients, 1/320 in 12 patients and 1/1000 in 3 patients with hydatid disease. Indirect immunofluorescence assay was positive in 10 of 15 patients with hepatic phase and in 3 of 7 patients with biliary phase of fascioliasis. The antibody titer was 1/100 in 6 and 1/320 in 7 patients with fascioliasis. The antibody titer was 1/100 in both healthy subjects. **Conclusions:** Indirect immunofluorescence assay for *Echinococcus granulosus* may be positive in a majority of patients with *Fasciola hepatica* infection and in some healthy subjects.

Key words: *Fasciola hepatica*, *Echinococcus granulosus*, indirect immunofluorescence assay

Fasciola hepatica enfeksiyonu olanlarda artmış anti-echinococcus granulosus antikor pozitifliği

Amaç: Helminter serolojik olarak çapraz reaksiyonlardan sorumlu olan bazı antijenlere sahiptirler. Bu çalışmanın amacı *Fasciola hepatica* enfeksiyonu tanısı alan hastalarda indirekt immunofloresan antikor (IFA) yöntemiyle *Echinococcus granulosus* antikorlarının sıklığını araştırmaktır. **Gereç ve yöntem:** Çalışmaya aşağıdaki gruplar alındı: *Fasciola hepatica* grubu (n=22), kist hidatik grubu (n=22) ve sağlıklı kontrol grubu (n=24). İndirekt immunofloresan antikor testi tüm vakalarda çalışıldı. **Bulgular:** İndirekt immunofloresan antikor testi kist hidatik hastalığı olan tüm vakalarda, fasiolazis hastalığı olan 22 vakanın 13 (%59)'ünde ve sağlıklı kontrol grubundaki 24 vakanın 2 (%8)'sinde pozitif. İndirekt immunofloresan antikor pozitiflik oranı kist hidatik grubunda *Fasciola hepatica* grubuna göre (<0.001) ve kontrol grubuna göre (p<0.001) anlamlı olarak daha yüksekti. İndirekt immunofloresan antikor pozitifliği *Fasciola hepatica* grubunda kontrol grubuna göre anlamlı olarak daha yüksekti (p=0.001). Antikor titresi kist hidatik hastalığı olan 7 vakada 1/100, 12 vakada 1/320 ve 3 vakada 1/1000 düzeyindeydi. Hepatik evre fasiolazisi olan 15 vakanın 10'unda ve biliyer evre fasiolazisli olan 7 vakanın 3'ünde indirekt immunofloresan antikor testi pozitif. Antikor titresi fasiolazisli 6 vakada 1/100 ve 7 vakada 1/320 düzeyindeydi. Kontrol grubundaki her iki şahısta da antikor titresi 1/100 düzeyinde saptandı. **Sonuç:** *Echinococcus granulosus* için yapılan indirekt immunofloresan antikor testi *Fasciola hepatica* enfeksiyonu olan vakaların çoğunda ve sağlıklı bireylerin bir kısmında pozitif olabilir.

Anahtar kelimeler: *Fasciola hepatica*, *Echinococcus granulosus*, indirekt immunofloresan antikor

INTRODUCTION

Fascioliasis is an infection caused by a trematode of the liver, *Fasciola hepatica*, which particularly affects sheep, goats and cattle. Human fascioliasis

is uncommon, and involvement of the liver is rare (1). Liver infection involves two stages - hepatic and biliary (1-3). Common signs and symptoms of

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the hepatic phase are abdominal pain, fever, eosinophilia, and abnormal liver function tests (1,2,4). The biliary phase usually presents with intermittent right upper quadrant pain with or without cholangitis or cholestasis (5,6). Diagnosis may be delayed because of the wide spectrum of the differential diagnosis and low incidence of *F. hepatica* infection (7). Similar abnormal laboratory and radiological findings may represent viral hepatitis, liver abscess, malignancy, cholecystitis, sclerosing cholangitis, acquired immunodeficiency syndrome (AIDS)-related cholangitis, ruptured hydatid cyst, and parasites such as ascariasis and clonorchiasis (1,3,4).

Human hydatid disease is caused by the larval form of the tapeworm genus *Echinococcus*. The most common form cystic hydatid disease results from infection with *Echinococcus granulosus* (EG). Although hydatid disease can develop anywhere in the human body, the liver is the most frequently involved organ (8). Hepatic hydatid disease causes highly variable symptoms and signs, and can be found incidentally in an asymptomatic patient. The symptoms and signs may be caused by a toxic reaction to the parasite or by local and mechanical effects, depending on the location and nature of the cysts and the presence of complications. The clinical course of hepatic hydatid disease is nonspecific and varied. The diagnosis of hydatid disease is based on clinical findings, imaging techniques and serology (9). The indirect immunofluorescence assay (IFA) is specific and sensitive, especially in cases of hepatic hydatidosis. IFA is the most sensitive test in more than 95% of patients with hepatic cystic hydatidosis (10).

The cross-reactivity between EG and *F. hepatica* infection has been reported previously (11-13). Parasitic helminths express some antigen, which often accounts for serological cross-reactions. In the serodiagnosis, it is essential to inspect cross-reactivity between the target parasite and other parasites in order to assess diagnostic performance (13).

The aim of this study was to determine the prevalence of anti-EG antibody in patients with *F. hepatica* infection.

MATERIALS AND METHODS

Study Population

This prospective study was conducted in the Gastroenterology and General Surgery Departments

of Dicle University Hospital between January 2008 and December 2010. The study population consisted of the following groups: *F. hepatica* group, hydatid disease group and control group. All patients gave written informed consent, and the study was approved by the local ethics committee.

In all subjects, an initial complete clinical history, physical examination findings, and routine laboratory results including complete blood count and routine biochemical analysis were recorded. Contrast-enhancement abdominal computerized tomographic (CT) examination was performed in all patients with *F. hepatica* infection and hydatid disease. Abdominal ultrasound (US) examination was performed in all patients in the control group. All the CT scans were obtained using a four-channel multislice CT scanner (Sensation 4, Siemens Medical Solutions, Erlangen, Germany). A 3.75-MHz convex probe (Toshiba SSA-270 A, Tokyo, Japan) was used for US of the abdomen.

A specific indirect hemagglutination assay using purified adult *F. hepatica* F1 antigen (IHA, Laboratoires Fumouze Diagnostic, Levallois Perret, France; cut-off 1:320) was used for serological diagnosis of fascioliasis. The diagnosis of *F. hepatica* infection with hepatic phase was based on: (a) The presence of previously described characteristic findings on the abdominal CT examination (3,14) and exclusion of all known diseases that cause hepatic lesion seen on CT examination; (b) the positive specific IHA for *F. hepatica*; (c) and/or the presence of eggs of *F. hepatica* in the fecal examination. The diagnosis of *F. hepatica* infection with biliary phase was based on the extraction of living *F. hepatica* during endoscopic retrograde cholangiopancreatography (ERCP).

The IFA for EG was performed using frozen sections of EG larvae (Euroimmun, Medizinische Labordiagnostika AG kit; cut-off 1/100) to determine the presence of IgA, IgG or IgM antibody in all subjects as previously described (10). The diagnosis of hydatid disease was confirmed by characteristic CT findings before surgery (8,9) and typical hydatid cystic appearance during surgery.

Patients who were followed for routine check-ups and were without any disease were included in the study as the control group.

Statistical Analysis

Comparison of laboratory features, age and gender between groups was done. Results are expressed as mean \pm standard error (SE) (range) or num-

ber (proportion) of patients. Chi-square test and Fisher's exact test were used for comparison of numerical values. Mann-Whitney U test was used for comparison of the mean of numerical values. P values were considered statistically significant at $p \leq 0.05$.

RESULTS

Table 1 shows the initial demographic and laboratory features of all groups. During the study period, 25 patients were diagnosed as *F. hepatica* infection. In 18 patients, the diagnosis of fascioliasis was based on positive IHA ($\geq 1/620$) test and characteristic abdominal CT findings. The mean titer of IHA for *F. hepatica* was $1/2720 \pm 549$ (range: 1/640-5120). These patients were accepted as the hepatic phase of fascioliasis. In the remaining 7 patients, diagnosis of fascioliasis was confirmed by extraction of living mobile *F. hepatica* from extrahepatic biliary ducts during the ERCP procedure. These patients were accepted as biliary phase of fascioliasis. Microscopic examination of fecal specimens for eggs of *F. hepatica* revealed a positive result in only 2 of 25 patients (1 biliary and 1 hepatic phase). After confirmation of fascioliasis, triclabendazole was administered at a dose of 10-12 mg/kg for 1 day in all patients. Six months after treatment, there was complete clinical and laboratory improvement in all patients. We excluded 3 patients with hepatic phase of fascioliasis from the study because of the absence of IFA kit for EG antibody during their admission to our hospital. The remaining 22 patients were included in the study. IFA for EG was positive in 10 of 15 (66%) patients with hepatic phase and in 3 of 7 (42%) patients with biliary phase. The antibody ti-

ter was 1/100 in 6 patients and 1/320 in 7 patients. The mean antibody titer was $1/129 \pm 29$ (range: 0-1/320).

There were 22 patients in the hydatid disease group. The hydatid cyst was located in the right lobe of the liver in 14 patients, left lobe in 3 patients, and both lobes in 4 patients and in the spleen in 1 patient. The number of cysts was one in 14 patients, two in 6 patients and three in 2 patients. According to the Gharbi classification (K-31), cysts were type II in 3 patients, type III in 11 patients, and type IV in 8 patients. The patient with splenic hydatid cyst had one type IV cyst. The mean cyst diameter was 9.63 ± 0.83 (range: 4-18) cm. IFA for EG antibody was positive in all patients. The mean antibody titer was $1/342 \pm 61$ (range: 1/100-1000). It was 1/100 in 7 patients, 1/320 in 12 patients and 1/1000 in 3 patients.

There were 24 subjects in the control group. Abdominal US showed no mass lesion in any of the patients. IFA for EG was positive in 2 female subjects. The antibody titer was 1/100 in both subjects.

Comparison of Groups

The positivity rate of IFA for EG was significantly higher in the hydatid disease group compared to the fascioliasis group (100% vs 59%, respectively; $p < 0.001$). Mean alanine aminotransferase (ALT), aspartate aminotransferase (AST) and eosinophil count were significantly higher in the *F. hepatica* group compared to the hydatid disease group ($p = 0.021$; $p = 0.026$; $p = 0.02$, respectively). There was no significant difference between the *F. hepatica* group and the hydatid disease group regarding white blood cell (WBC) count, alkaline phospho-

Table 1. Initial demographic and laboratory features of all groups

	Hydatid disease group	Fasciola hepatica group	Control group
Gender (M/F)	7/15	3/19	14/10
Age [†]	30 (15-80)	40 (19-79)	32 (15-77)
WBC (n/mm ³) [*]	9694±766	10323±742	7656±535
Eosinophil [†] (% of total WBC)	4 (0.1-33)	25.3 (0.8-68)	1.5 (0.9-9)
ALT [*]	47±12	98±32	25±5
AST [*]	38±7	98±47	25±5
ALP [*]	138±45	163±15	69±5
GGT [*]	128±76	109±22	33±4
T. bilirubin [*]	0.82±0.33	0.84±0.24	0.64±0.06
Positivity rate of IFA for EG (%)	100	54.5	8.3

[†]: value expressed as median (range), ^{*}: value expressed as mean±standard error. ALT: Alanine aminotransferase (range:10-40 U/L). AST: Aspartate aminotransferase (range: 10-35 U/L). GGT: Gamma glutamyl transferase (range: 9-64U/L). ALP: Alkaline phosphatase (range: 40-150 U/L). T. bilirubin: Total bilirubin (range: 0.2-1.2 mg/dl). IFA: Indirect immunofluorescence assay. EG: *Echinococcus granulosus*.

hatase, gamma glutamyl transferase (GGT), and total bilirubin level.

The positivity rate of IFA for EG was significantly higher in the hydatid disease group compared to the control group (100% vs 8.3%; $p < 0.001$). There was no significant difference between the hydatid disease group and the control group regarding total WBC count, ALT, AST, GGT, and total bilirubin level. Mean alkaline phosphatase and eosinophil count were significantly higher in the hydatid disease group compared to the control group ($p = 0.026$ and $p = 0.008$, respectively).

The positivity rate of IFA for EG was significantly higher in the *F. hepatica* group compared to the control group (59% vs 8.3%; $p = 0.001$). Total WBC count, eosinophil count, ALT, AST, alkaline phosphatase, and GGT level were significantly higher in the *F. hepatica* group compared to the control group ($p < 0.001$ for all parameters). There was no significant difference between the *F. hepatica* group and the control group regarding total bilirubin level.

DISCUSSION

Parasitic helminths express various antigenic carbohydrates, which often account for serological cross-reactions. In the serodiagnosis, it is essential to inspect cross-reactivity between the target parasite and other parasites in order to assess diagnostic performance. The Gal (β 1-6) Gal sequence is a common epitope between EG and *F. hepatica* (13). Wuhler *et al.* (15) reported that *F. hepatica* exhibits mammalian-type glycolipids as well as Gal (β 1-6) Gal-terminating glycolipids that account for cestode serological cross-reactivity. Ramzy *et al.* (12) reported that 5% of healthy people have IgG3 antibody and 10% of healthy people have IgG4 antibody for EG. Cross-reactivity has been observed with total IgG antibody followed by that of IgG4 in the sera from cases with trichostrongyliasis, ancylostomiasis, schistosomiasis, ascariasis, and fascioliasis. In this study, we showed that IFA for EG was positive in 59% of patients with fascioliasis and 8.3% of healthy people. All these positive results were in low titers ($\leq 1/320$). The high incidence of positive IFA test for EG in patients with fascioliasis may be related to the antigenic similarity between *F. hepatica* and EG, but positive results in healthy people may be related to past exposure to EG. The high incidence of cross-reactivity especially in low titers between parasites can suggest that serological tests without additional confirma-

tory tests such as characteristic radiological findings are not a reliable method for diagnosis of these infections. The important limitation of this study is that we did not investigate the presence of antibody titers of *F. hepatica* in the patients with hydatid disease. The presence of common antigens in *F. hepatica* and EG may also suggest the presence of antibody against *F. hepatica* in EG infection.

Typical organ lesion(s) detected by imaging techniques (e.g. US, CT), specific serum antibodies assessed by high-sensitivity serological tests, histopathology or parasitology compatible with EG, and detection of pathognomonic macroscopic morphology of cyst(s) in surgical specimens confirm the diagnosis of EG (9). Routine laboratory tests are not specific for the diagnosis of hydatid disease and may reveal normal or abnormal values. Screening tests such as IHA, enzyme-linked immunosorbent assay (ELISA) and latex agglutination using crude antigens are associated with a high incidence of false-negative and false-positive results (8). The parasitic antigens of major diagnostic value are antigen 5 (arc-5) and antigen B (8,16). Purified fractions enriched in antigens 5 and B and glycoproteins from hydatid fluid yielded a sensitivity rate of 95%, with a specificity rate of 100% (17). The diagnosis of hydatid disease was confirmed in all our patients by positive IFA test, CT findings and pathognomonic surgical findings.

Diagnosis of fascioliasis may be delayed because of the wide spectrum of the differential diagnosis and low incidence of *F. hepatica* infection (3). Similar abnormal laboratory and radiological findings may represent viral hepatitis, liver abscess, malignancy, cholecystitis, sclerosing cholangitis, AIDS-related cholangitis, ruptured hydatid cyst, and parasites such as ascariasis and clonorchiasis (1,3). The specificity of the IHA method using purified adult *F. hepatica* antigen F1 for serological diagnosis of *F. hepatica* is 96.9% (18). Diagnosis is confirmed only by demonstrating the parasites or its egg in the bile or feces (1,3). Negative stool examinations do not rule out the disease (3,7). A high index of suspicion and specific radiological findings including tunnel-like tracts extending towards the capsule and multiple, hypodense, linear or branching lesions on CT are very helpful in the diagnosis of fascioliasis. We suspected the possibility of fascioliasis in all patients with hepatic phase because of eosinophilia and characteristic CT findings. We found eggs in the stool sample of 1 of 16 patients with hepatic phase and 1 of 7 patients with biliary phase. Complete clinical, laboratory

and radiological response after triclabendazole administration associated with positive result in high titer of IHA against *F. hepatica* confirmed the diagnosis in patients with hepatic phase of *F. hepatica* infection. Diagnosis in the patients with biliary phase was confirmed by extraction of living *F. hepatica* from bile ducts.

In conclusion, cross-reactions are an important issue in the serological diagnosis of parasitic infections. IFA for EG is positive in the majority of patients with *F. hepatica* infection and in some healthy subjects. In clinical practice, IFA for EG is not a reliable serological diagnostic method for differentiation of hydatid disease from fascioliasis.

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