Original Article

Clinical Features, Diagnosis and Management of Patients with Suspicions of Fascioliasis in Kohgiluyeh and Boyer-Ahmad Province, Southwestern Iran

Abdolali MOSHFE 1, Arash ARIA 1, Najme ERFANI 1, Ali JAMSHIDI 1, *Bahador SARKARI 2,3, Samaneh ABDOLAHI KHABISI 4, Nasir AREFKHAY 1

1. Cellular and Molecular Research Center, Yasuj University of Medical Sciences, Yasuj, Iran
2. Department of Parasitology and Mycology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran
3. Basic Sciences In Infectious Diseases Research Center, Shiraz University of Medical Sciences, Shiraz, Iran
4. Department of Parasitology and Mycology, School of Medicine, Zahedan University of Medical Sciences, Zahedan, Iran

Received 04 Mar 2019
Accepted 12 Jun 2019

Keywords: Clinical features; Diagnosis; Treatment; Fasciolosis; Iran

Abstract

Background: In the current study, we described the epidemiological features, clinical presentation, diagnosis and management of patients with suspicion of fascioliasis in Kohgiluyeh and Boyer-Ahmad Province in southwest of Iran.

Methods: Overall, 56 patients with suspicion of fascioliasis, based on their clinical signs and symptoms that referred to Clinic of Internal Medicine in Yasuj city, from 2014 to 2016 were enrolled. Demographic data, history of eating aquatic local plants, the chief complains, and laboratory findings were recorded for each patient. Stool samples were obtained from each case for detection of Fasciola eggs. Moreover, blood samples were taken from each patient and evaluated for detection of anti-Fasciola antibodies by an indirect ELISA. Patients who defined as having fascioliasis were treated with triclabendazole and were followed for at least three months for clinical improvement.

Results: Serological test was positive in 5 patients. Of these 5 cases, three cases had a history of ingesting raw aquatic vegetables. The main clinical signs and symptoms in positive cases were; abdominal pain (60%), epigastric pain (40%), anemia (60%), and dermal pruritus (20%). Hypereosinophilia was seen in all of 5 positive cases. No Fasciola egg was found in stool specimens of any of the patients. The fascioliasis cases were treated by triclabendazole and clinical symptoms disappeared in all of 5 cases.

Conclusion: Our observation further confirmed Yasuj district as a human endemic area for fascioliasis in Iran. The study also highlighted the importance of clinical features together with eosinophilia, as key parameters, in the diagnosis of human fascioliasis. Clinicians need to be aware of this disease and should keep in mind fascioliasis when hypereosinophilia present in patients in such endemic areas.
Introduction

Fascioliasis caused by liver flukes Fasciola hepatica and F. gigantica via ingestion of aquatic plants contaminated with encysted metacercarial stage of the worm (1). Although human fascioliasis is frequently reported from Andean and some of European countries, the highest prevalence of human fascioliasis with relatively high annual cases have been reported from a few countries, including Egypt, Iran, Peru and Bolivia (1-5).

Human fascioliasis has two distinct clinical phases; the time of migration of juvenile fluke and when the adult worm get into the bile duct and settle. A symptomless incubation period, lasting for a few days to a few months, starts once the larvae are ingested with contaminated aquatic plants and followed by an acute and a chronic clinical phase. The acute phase starts when the immature worms are migrating through the liver. The juvenile flukes puncture the liver's surface and move around until they reach the bile ducts. This invasion is accompanied by a swollen liver, skin rashes and extreme abdominal pain (6, 7). The chronic phase begins when the worms reach the bile ducts, which cause intermittent pain, cholangitis, obstructive jaundice, and eosinophilia (6). Diagnosis of fascioliasis relies on its clinical features along with laboratory methods (8-12).

Previous studies demonstrated a new focus of human fascioliasis in Yasuj district in Kohgiluyeh and Boyerahmad Province, southwest of Iran, where animal fasciolosis is quite common (4, 5, 13, 14). Both F. hepatica and F. gigantica are present in animals in this area (13-15). Human infection has been confirmed in some patients by parasitological (detection of eggs in stool samples), serological (ELISA and western blotting) and molecular (PCR and sequencing) approaches in this area (4, 5, 8). Molecular studies demonstrated F. hepatica as the causative agent of human fascioliasis in few of the patients in the area (4).

Nowadays, physician awareness about human fascioliasis in this human endemic area of fascioliasis has increased and during the last 10 years, progress has been made in understanding the clinical features of fascioliasis. Therefore, much more cases with suspicion of fascioliasis have been referred to the university-affiliated health centers for proper diagnosis and management by general practitioners, infectious disease specialist as well as internist.

Both ELISA and western blotting have been used for diagnosis of suspected cases in the area. The sensitivity and specificity of serological methods, using ELISA based on Fasciola excretory-secretory (ES) antigens exceed 95% (12). Results of serological testing may become positive 2–4 weeks after infection, preceding the presence of eggs in the stool.

Eosinophilia is more likely to be present during the parenchymal phase; however, the eosinophil count may be normal in up to 50% of chronic cases. Normal eosinophil count cannot be used to exclude parasitic etiology (16). On the other hand, stool microscopy is not conclusive for the diagnosis of human fascioliasis in the acute phase of illness, as the pre-patent period (time from infection to shedding of ova in the feces by mature adult worms) is around four months (10).

In this study, we described the epidemiological features, clinical presentation, diagnosis and management of patients with suspicion of fascioliasis, misdiagnosed with other diseases, in Kohgiluyeh and Boyer-Ahmad Province, Southwestern Iran. Moreover, we highlighted the importance of clinical presentation and diagnostic parameters of fascioliasis, underlined the significant role of eosinophilia in the diagnosis of human fascioliasis.

Materials and Methods

Overall, 56 patients with suspicion of fascioliasis, based on their clinical signs and symp-
toms that referred to Clinic of Internal Medicine, a university-affiliated clinic, in Yasuj City, Southwestern Iran during Feb 2014 to Sep 2016 were enrolled in this study. While patients were being seen by the internist, demographical data, history of eating aquatic local plants, the chief complains, clinical presentation and the available laboratory findings (mainly eosinophilia) were recorded. Stool samples were obtained from each case in three subsequent times for detection of *Fasciola* spp. eggs. Moreover, 5 mL of fresh blood was taken from each patient for serological evaluation of fascioliasis. Each stool sample was examined by concentration method (Formalin Ether). Sera samples were sent to the Department of Parasitology and Mycology, at School of Medicine in Shiraz University of Medical Sciences (SUMS) for detection of anti-*Fasciola* antibodies by an indirect ELISA, using excretory/secretory antigens of *F. hepatica* (8, 17). Patients who defined as having fascioliasis, based on a positive serological test and conclusive signs and symptoms, were treated with triclabendazole (2 individual doses of 10 mg/kg, separated in time by 24 h). Treated patients were followed for at least 3 months for recovery and improvement of the symptoms. Criteria for improvement were disappearance of main symptoms of fascioliasis and reduction in blood eosinophilia (less than 450/mm).

The study was approved by Ethical Committee of Yasuj University of Medical Sciences (YUMS) and informed consent was obtained from each participant before enrolling in the study.

All the collected data were analyzed, using SPSS software (ver. 18) (Chicago, IL, USA).

**Results**

Of 56 clinical suspicion cases of fascioliasis, 5 (8.9%) female patients were positive by ELISA test. Of these cases, 4 patients were above 50 yr old and 3 cases had a history of ingesting raw aquatic vegetables (locally named, Bakaloo). The main clinical signs and symptoms in positive cases were; abdominal pain in 3, epigastric pain in 2, anemia in 3 cases and dermal pruritus and nausea each in 1 patient. Hypereosinophilia was seen in all of 5 positive cases. No *Fasciola* eggs were found in stool specimens of any of the patients. Obstructive jaundice was not seen in any of the positive cases. The positive cases were treated by triclabendazole and clinical symptoms disappeared in all of 5 cases when followed for 3 months after treatment. Table 1 shows the age distribution of patients’ suspicion of fascioliasis.

| Age (yr) | Serological assay (ELISA) | Total |
|---------|---------------------------|-------|
|         | Negative | Percent | Positive | Percent | No. | Percent |
| ≤ 10    | 8 | 14.3 | 0 | 0 | 8 | 14.3 |
| 11-20   | 7 | 12.5 | 0 | 0 | 7 | 12.5 |
| 21-30   | 11 | 19.6 | 1 | 1.8 | 12 | 21.4 |
| 31-40   | 6 | 10.7 | 0 | 0 | 6 | 10.7 |
| 41-50   | 11 | 19.6 | 0 | 0 | 11 | 19.6 |
| 51-60   | 5 | 8.9 | 1 | 1.8 | 6 | 10.7 |
| ≥ 60    | 3 | 5.4 | 3 | 5.4 | 6 | 10.8 |
| Total   | 51 | 91.1 | 5 | 8.9 | 56 | 100 |
Discussion

Fascioliasis is an important helminth infection caused by *F. hepatica* and *F. gigantica* (1). Human is an accidental host and can be infected through ingestion of metacercaria-carrying aquatic plants or contaminated water. The main clinical manifestation of human fascioliasis are the abdominal pain mainly in right upper quadrant, nausea, vomiting, skin rash, itching, obstructive jaundice, cholangitis, and hypereosinophilia. Clinical suspicion of fascioliasis may arise in patients complaining of right upper quadrant pain, fever, anorexia, and jaundice. Moreover, the presence of eosinophilia along with a history of ingestion of aquatic plants in endemic areas may lead to the suspicion of fascioliasis.

Human fascioliasis is a considerable health problem in Iran, especially in the north of the country where two biggest outbreaks of human fascioliasis in the world have occurred during the last decades (2, 3).

Yasuj is a mountainous district in the southwest of Iran, which has recently been introduced as a new focus of human fascioliasis (4, 5). The unique climate condition of this region favored the spread of numerous parasitic diseases (4, 5, 18-20). In this area, fascioliasis is probably transmitted to human by the use of freshwater plants such as *Nasturtium microphyllum* (local name, Bakaloo) and *Menthe logifolia* (local name, Pooneh). The disease is also quite common in livestock in the area (13, 14, 21). Both *F. hepatica* and *F. gigantica* have been isolated from animals in the region while the species responsible for human fascioliasis is reported to be *F. hepatica* (4, 5, 13).

In the current study fascioliasis patients were complaining of abdominal pain (40%), itching (20%) and nausea (20%). Similar clinical features have been reported in a familial outbreak of 24 fascioliasis patients in eastern Anatolia, in Turkey (22).

In our study, *Fasciola* eggs were not found in the stool specimens of any of the patients. The absence of eggs in the stool samples of positive cases may be due to the inability of *Fasciola* to produce eggs, lack of adaptation of the fluke to the human host, or encapsulation of eggs in the liver granuloma, and low egg releasing because of low infection burden or old infection (12). Furthermore, intermittent egg production and cessation of egg shedding in the advanced chronic phase of fascioliasis is common. Failure to find eggs in the patient’s stools may also be due to the disease being in the acute phase. Besides, the biliary obstruction can also be one of the causes of the lack of eggs in the patient’s stool sample. In keeping with our findings, none of the serologically proven fascioliasis cases in an outbreak of human fascioliasis in Kermanshah in the western part of Iran was egg positive (23). Similar findings have been described in a case series of fascioliasis in Australian travelers to Bali, where 6 cases of fascioliasis have been reported and stool microscopy have been negative in all of 6 cases (24). These observations are consistent with our findings. In a study of 711 fascioliasis cases in France during a 30-year epidemiological survey, only 27.6% of cases were egg-positive (25). Moreover, in a series of 23 cases of serologically and clinically proven human fascioliasis in Egypt, only two cases (8.7%) were egg positive (26).

Serological diagnosis, based on antibody detection, is an appropriate approach for the diagnosis of fascioliasis. This is mainly because the patient usually presents clinical signs or symptoms long before than the egg appears in the stool, whereas antibodies to *Fasciola* antigens can be detected in patient’s sera two weeks after infection, long before the beginning of egg shedding (10). In our study, an ELISA system, based on ES antigens of *F. hepatica*, was applied for the serological diagnosis of fascioliasis cases. This ELISA system has appropriate performance (more than 95% sensitivity and specificity), in the diagnosis of human fascioliasis (12).
Marked eosinophilia is one of the hallmarks of acute fascioliasis and in the endemic areas; it should raise the suspicion of fascioliasis. In our cases, blood eosinophilic counts were considerably high in all of the 5 patients. Eosinophilia is a key feature of fascioliasis, which is present in most of the fascioliasis patients. Eosinophilia can be seen during parenchymal phase as well as in the chronic phase (24). In the case series of 24 fascioliasis patients in Anatolia, eosinophilia was seen in 70% of the cases. The authors concluded that eosinophilia is an important predictor for fascioliasis in the patient with consistent clinical manifestations (22). Eosinophilia disappears after successful treatment and if remains it can be a clue for treatment failure.

Triclabendazole is considered the drug of choice for treatment of human fascioliasis. It is a benzimidazole derivative with a cure rate of around 80% with the first course and almost 90-100% with the second course of treatment. The drug has been successfully used for the treatment of human fascioliasis in the two major outbreaks of fascioliasis in Iran (3). In our study, clinical improvement was seen in all of the fascioliasis patients when treated with a double dose of triclabendazole.

Taken together, our observation further confirmed Yasuj district as a human endemic area for fascioliasis in Iran. The study also highlighted the importance of clinical features together with eosinophilia as key parameters in the diagnosis of human fascioliasis in such endemic areas. Clinicians need to be aware of this disease and should keep in mind fascioliasis when hypereosinophilia present in patients in such endemic areas.

Conclusion

Yasuj district should be regarded as a human endemic area for fascioliasis in Iran. We highlighted the importance of clinical features together with eosinophilia, as key parameters, in the diagnosis of human fascioliasis. Clinicians need to be aware of this disease and should keep in mind fascioliasis when hypereosinophilia present in patients in such endemic areas.

Acknowledgements

The study was the subject of Mrs. Najme Erfani MD thesis.

Financial support

The study was financially supported by the office of vice-chancellor for research of Yasuj University of Medical Sciences.

Conflict of interest

The authors declare that they have no competing interests.

References

1. Mas-Coma S, Valero MA, Bargues MD. Chapter 2. Fasciola, lymnaeids and human fascioliasis, with a global overview on disease transmission, epidemiology, evolutionary genetics, molecular epidemiology and control. Adv Parasitol. 2009; 69:41-146.
2. Ashrafi K, Bargues MD, O'Neill S, Mas-Coma S. Fascioliasis: a worldwide parasitic disease of importance in travel medicine. Travel Med Infect Dis. 2014;12:636-649.
3. Ashrafi K, Saadat F, O'Neill S et al. The endemicity of human fascioliasis in Guilan Province, Northern Iran: the baseline for
implementation of control strategies. Iran J Public Health. 2015; 44:501-511.

4. Hosseini G, Sarkari B, Moshfe A et al. Epidemiology of human fascioliasis and intestinal helminthes in rural areas of Boyer-Ahmad Township, Southwest Iran; A population based study. Iran J Public Health. 2015; 44:1520-1525.

5. Sarkari B, Ghobakhloo N, Moshfe A, Eilami O. Seroprevalence of human fascioliasis in a new-emerging focus of fasciolosis in Yasuj district, southwest of Iran. Iran J Parasitol. 2012; 7:15-20.

6. Haseeb AN, El-Shazly AM, Araf MA, Morsy AT. Clinical, laboratory and ultrasonography features of proven human fascioliasis. J Egypt Soc Parasitol. 2003; 33:397-412.

7. Karadag-Oncel E, Oszurekci Y, Ozkaya-Parlakay A et al. *Fasciola hepatica* infection: clinical and radiological findings in pediatric patients. Turk J Pediatr. 2012; 54:362-367.

8. Shafiei R, Sarkari B, Sadjjadi SM. Performance of a 27 kDa *Fasciola hepatica* antigen in the diagnosis of human fascioliasis. J Lab Physicians. 2015; 7:17-20.

9. Lukambagire AH, McHaile DN, Nyindo M. Diagnosis of human fascioliasis in Arusha region, northern Tanzania by microscopy and clinical manifestations in patients. BMC Infect Dis. 2015; 15:578.

10. Mas-Coma S, Bargues MD, Valero MA. Diagnosis of human fascioliasis by stool and blood techniques: update for the present global scenario. Parasitology. 2014; 141:1918-1946.

11. Abdolahi Khabisi S, Sarkari B, Moshfe A, Jalali S. Production of monoclonal antibody against excretory-secretory antigen of *Fasciola hepatica* and evaluation of its efficacy in the diagnosis of fascioliasis. Monoclon Antib Immunodiagn Immunother. 2017; 36:8-14.

12. Sarkari B, Khabisi SA. Immunodiagnosis of human fascioliasis: an update of concepts and performances of the serological assays. J Clin Diagn Res. 2017; 11:0e05-o10.

13. Sarkari B, Parhoode M, Abdolahi Khabisi S et al. Genetic diversity of *Fasciola* spp. isolates from northern part of Iran: comparison with southwestern isolates. J Parasit Dis. 2017; 41:768-772.

14. Shafiei R, Sarkari B, Sadjjadi SM et al. Molecular and morphological characterization of *Fasciola* spp. isolated from different host species in a newly emerging focus of human fascioliasis in Iran. Vet Med Int. 2014; 2014:405740.

15. Shafiei R, Sarkari B, Moshfe A. A Consistent PCR-RFLP assay based on ITS-2 ribosomal DNA for differentiation of *Fasciola* species. Iran J Basic Med Sci. 2013; 16:1266-1269.

16. Oner Vatan A, Mete B, Yemisen M et al. A case of *Fasciola hepatica* mimicking sepsis without eosinophilia. Turkiye Parazitol Derg. 2014; 38:131-134.

17. Abdolahi Khabisi S, Sarkari B. Detection of Fasciola hepatica and Fasciola gigantica common and uncommon antigens, using rabbit hyper immune serum raised against their excretor-secretory and somatic antigens. J Parasit Dis. 2016; 40:1552-1557.

18. Sarkari B, Hosseini G, Motazedian MH et al. Prevalence and risk factors of intestinal protozoan infections: a population-based study in rural areas of Boyer-Ahmad district, Southwestern Iran. BMC Infect Dis. 2016; 16:703.

19. Sarkari B, Pedram N, Mohebali M et al. Seroepidemiological study of visceral leishmaniasis in Booeyerahmad district, southwest Islamic Republic of Iran. East Mediterr Health J. 2010; 16:1133-1136.

20. Sarkari B, Sadjjadi SM, Beheshtian MM, et al. Human cystic echinococcosis in Yasuj District in Southwest of Iran: an epidemiological study of seroprevalence and surgical cases over a ten-year period. Zoonoses Public Health. 2010; 57:146-150.

21. Abdolali M, Rezaei Nasrabad SA, Cheraghzadeh SR, et al. Study on prevalence of fascioliasis in ruminants in Dasht Room County in spring and summer of 2013. Animal Vet Sci. 2016; 4:15-18.

22. Karahocagil MK, Akdeniz H, Sunnetcioglu M et al. A familial outbreak of fascioliasis in Eastern Anatolia: a report with review of literature. Acta Trop. 2011; 118:177-183.

23. Hatami H, Asmar M, Masoud J et al. The first epidemic and new-emerging human fascioliasis in Kermanshah (western Iran) and a ten-year
follow up, 1998-2008. Int J Prev Med. 2012; 3:266–272.

24. Figtree M, Beaman MH, Lee R et al. Fascioliasis in Australian travellers to Bali. Med J Aust. 2015; 203:186-188.

25. Rondelaud D, Dreyfuss G, Vignoles P. Clinical and biological abnormalities in patients after fasciolosis treatment. Med Mal Infect. 2006; 36:466-468.

26. Mekky MA, Tolba M, Abdel-Malek MO, Abbas WA, Zidan M. Human fascioliasis: a re-emerging disease in upper Egypt. Am J Trop Med Hyg. 2015; 93:76-79.

27. Ashrafi K, Tahbaz A, Sharifdini M, Mas-Coma S. Familial Trichostrongylus infection misdiagnosed as acute fascioliasis. Emerg Infect Dis. 2015; 21(10):1869-70.