Fascioliasis and COVID-19: A Case-Control Study in Iran

Fatemeh Balazadeh
Alborz University of Medical Sciences

Mohammad Zibaei (✉ zibaeim@sums.ac.ir)
Alborz University of Medical Sciences

Aliehsan Heidari
Alborz University of Medical Sciences

Hadis Rastad
Alborz University of Medical Sciences

Farzaneh Firoozeh
Alborz University of Medical Sciences

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Abstract

**Background:** Human Fascioliasis is a zoonotic infection caused by the adult stage of *Fasciola* species. *Fasciola hepatica* is a parasite that, following living in its vertebrate host, reduces the host’s inflammatory immune responses to infectious diseases, including COVID-19. Therefore, because of the completely unproven and limited data on this issue, we decided to evaluate the prevalence of antibodies against this parasite in patients admitted to the hospital due to relatively severe symptoms of COVID-19.

**Methods:** This cross-sectional study was conducted on the serum samples of 182 patients with COVID-19 disease who were diagnosed by PCR test and 210 healthy subjects in Alborz Province, Iran between March and June 2020. Anti-*Fasciola* antibodies status was determined in all serum samples, using ELISA technique. A self-developed questionnaire was used to measure demographic information.

**Results:** The frequency of *Fasciola* infection was found to be significantly higher in COVID-19 patients as compared to healthy control (n = 4, 2.2%, n = 9, 4.3%; respectively) \((P = 0.041)\). There was no significant difference between COVID-19 patients and control group in age, education, and gender \((P > 0.05)\).

**Conclusions:** This study indicates that a significant association between *Fasciola hepatica* seropositivity and COVID-19 disease. Our finding suggests that *Fasciola* infection may be able to lead to decreased immune responses, thereby reducing the severity and symptoms of COVID-19 disease.

**Background**

Fascioliasis is widespread, zoonotic parasitic disease caused by the adult stages of either *Fasciola hepatica* or *Fasciola gigantica* [1]. *Fasciola* infection is common in many parts of the world, especially in areas where livestock is more prevalent [2–4]. Although fascioliasis is recognized as a significant veterinary problem, human fascioliasis has become an important health issue in many countries around the world [5, 6]. According to the World Health Organization (WHO), Iran is among the six most infected countries in the world with serious concerns about *Fasciola* infection [7]. Fascioliasis has led to two major epidemics in Iran in 1989 and 1999, respectively, which have been the largest outbreaks of fascioliasis in history [8]. It has been shown that the serological tests are commonly used to detect anti-*Fasciola* antibodies in acute-phase serum specimens and ectopic fascioliasis [9–11].

Parasites can suppress host immune responses. They suppress some of the pathways involved in activating immune responses, such as the presentation of antigens by dendritic cells, the production of cytokines by T cells, the production of antibodies by B cells, and the release of alarming by epithelial cells [12]. They also modulate other pathways, such as differentiation of Th cell subsets and B cell isotype changes, as well as inducing other pathways such as Treg cells and Breg cells differentiation and tolerant responses of dendritic cells. Further details are under investigation. As more information becomes available on how anti-parasitic responses are produced, scientists are also discovering new ways to modulate the immune system by these parasites and use parasitic molecules as treatments for inflammatory diseases [12].
Coronaviruses are in the Orthocoronavirinae subfamily, Coronaviridae family, and Nidovirales order. In the Orthocoronavirinae subfamily, there are four species of alpha (α), beta (β), gamma (γ), and delta (δ) coronavirus. Alpha and beta types are pathogenic in mammals while gamma and delta types in birds [13]. The coronavirus 2019, known as COVID-19, which was first identified in Wuhan, China, and quickly spread to other parts of the world, caused a recent pandemic in the world that led to the infection and death of many people around the world [13]. A report indicates that out of 425 patients studied in Wuhan; about 55% had a history of work in seafood wholesale market. Therefore, it has been shown that the disease can be transmitted from animals to humans [14]. Due to the importance of this helminthic zoonosis and the lack of data about its prevalence in our region, the high rate of migration to it, the report of COVID-19 patients, the prevalence of cattle infection in the region, and the study of new foci of fascioliasis in the our study area [15, 16], the aim of current was to investigate fascioliasis among the COVID-19 patients and the epidemiological factors associated with the infection and compare them with a healthy population in Iran.

Methods

Study Area

Alborz Province centered in Karaj (35°49′57″, 50°59′29″), is located in the north of Iran, bordering with the states of Tehran, Qazvin, Markazi, and Mazandaran (Fig. 1). This region with a population of around 3 million is one of the five most populous cities in Iran. The Alborz Province climate is to some extent cooler than the neighboring provinces; this area has a 15.1°C mean temperature and receives 260 mm of rain annually (https://en.wikipedia.org/wiki/Alborz_Province).

Study design

The demographic details of the participants and data relating to their demographic characteristics including age, gender, place of residence, occupation, history of disease, and level of education prior the sampling and testing were recorded, after obtaining ethical approval.

Subjects

This cross-sectional study was conducted on 182 patients (86 women and 96 men) with COVID-19 aged between 14 and 72 years [mean 55.17 (± 19.04)] who were followed up in the Infectious Diseases Division of Shahid Rejaei Hospital, as a referral hospital for patients with COVID-19, between March and June 2020. These patients had evaluation of their clinical history, imaging technique such as CT scans or serological examination. The diseases were defined accordingly to the PCR.

Control population

The control group consisted of 123 women and 87 men volunteers aged between 10 and 89 years [mean 30.29 (± 12.14)] with comparable epidemiological characteristic and without any complaints or history of
previous coronaviruses diseases 2019 in either themselves or their families.

**Serological examination**

Three mL venous blood was taken under sterile conditions from each subject in the both group and the sera of these blood samples were separated by centrifuge at 2500 rpm, aliquot, and store at -20°C until analyses were carried out. Anti-\textit{Toxocara} antibodies were detected by commercial ELISA kit (Pishtaz Medical Research Company, Tehran, Iran) following the manufacture’s instruction. Briefly, ELISA microplates have been coated with \textit{Toxocara canis} excretory-secretory antigens. First, serum samples were added to the plates and incubated at room temperature for 1 hour. The plates were washed again and diluted anti-human IgG horseradish peroxidase conjugated was added to each well and incubated for 1 hour at 37°C. After washing three times, the plates were incubated with chromogen/substrate and were stopped by addition stopper solution. The OD of samples was monitored at a wavelength of 450 nm using a microplate reader.

**Statistical analysis**

All epidemiological, clinical and laboratory information were tested for their association between with fascioliasis. Comparisons between quantitative variables were performed by T-test, nonparametric, and Mann-Whitney U tests. SPSS software (version 21) was used for statistical analysis of data and the significance level of \(P\)-value was less than 0.05.

**Results**

Out of 182 COVID-19 patients who participated in the study, 4 (2.2%) had positive anti-\textit{Fasciola hepatica} antibodies, as compared to 9 (4.3%) among healthy group. This was statistically significant \((P=0.041)\) as shown in Table 1.

|                           | COVID-19 patients | Control group | Total | \(P\)-value |
|---------------------------|-------------------|---------------|-------|-------------|
|                           | Number | Percent | Number | Percent |         |
| Seropositive              | 4      | 2.2     | 9      | 4.3      | 14      | 0.041  |
| Seronegative              | 178    | 97.8    | 201    | 95.7     | 378     |        |
| Total                     | 182    | 100     | 210    | 100      | 392     |        |

\(^a\)COVID-19

There was no significant difference between COVID-19 patients and control in terms man age, age group, and gender. On the other hand, there were more unemployed among the COVID-19 patients, whereas there were more workers among the control group (Table 2).
Table 2
Epidemiological and demographical characteristics of COVID-19 patients and control group

| Variable                  | COVID-19 patients | Control group | Total | Statistical (P-value) |
|---------------------------|------------------|---------------|-------|----------------------|
|                           | Number | Percent | Number | Percent |          |          |
| Age Categories (years)    |         |         |         |         |          |
| ≤ 19                      | 2       | 1.1     | 1       | 0.5      | 3        | 0.578    |
| 20–39                     | 39      | 21.4    | 20      | 9.5      | 59       |          |
| 40–59                     | 56      | 30.8    | 75      | 35.7     | 131      |          |
| 60–79                     | 66      | 36.3    | 87      | 41.4     | 153      |          |
| ≥ 80                      | 19      | 10.4    | 27      | 12.9     | 46       |          |
| Gender                    |         |         |         |         |          |
| Female                    | 86      | 47.3    | 123     | 209      | 0.262    |
| Male                      | 96      | 52.7    | 87      | 183      |          |
| Education                 |         |         |         |         |          |
| No School                 | 7       | 3.9     | 3       | 1.4      | 10       | 0.252    |
| Some high School          | 39      | 21.4    | 36      | 17.1     | 75       |          |
| High School               | 79      | 43.4    | 104     | 49.6     | 183      |          |
| Some college/Graduate school | 57    | 31.3    | 67      | 31.9     | 124      |          |
| Occupation                |         |         |         |         |          |
| Unemployed                | 100     | 54.9    | 39      | 18.6     | 139      | 0.416    |
| Worker                    | 15      | 8.2     | 51      | 24.3     | 66       |          |
| Housekeeper               | 34      | 18.8    | 45      | 21.4     | 79       |          |
| Farmer                    | 8       | 4.4     | 15      | 7.2      | 23       |          |
| Student                   | 15      | 8.2     | 36      | 17.1     | 51       |          |
| Other                     | 10      | 5.5     | 24      | 11.4     | 34       |          |
| Residency                 |         |         |         |         |          |
| Rural                     | 110     | 60.4    | 174     | 82.9     | 284      | 0.548    |
| Urban                     | 72      | 39.6    | 36      | 17.1     | 108      |          |

The seroprevalence rate in COVID-19 patients was substantially higher among the people aged 40–59 years (n = 2, 50.0%) than in older subjects (60–79 years, n = 1, 25.0%; and ≥80 years, n = 1, 25.0%) (P=...
0.067). High-titer antibodies were most prevalent among those in the age group 40–59 years, suggesting that recent or current exposure to *Fasciola* is most common in this age group. Seropositivity was more prevalent in females \( (n = 3, 75.0\%) \) than in males \( (n = 1, 25.0\%) \) related to COVID-19 and showed statistical significance \( (P< 0.05) \).

Based on the results of T-test, no significant difference was found between the seroprevalence of fascioliasis and COVID-19 patients due to educational levels \( (P = 0.091) \). So that, the seroprevalence of *Fasciola* infection in the patients with some high school \( (n = 2) \), high school \( (n = 1) \), and graduate school \( (n = 1) \) was not significant. There was significantly higher proportion of housekeeper \( (n = 3, 75.0\%) \) among the seropositive subjects than worker \( (n = 1, 25.0\%) \) \( (P = 0.045) \).

There was predominance of rural compared to urban population among our seropositive COVID-19 patients. Of the 4 (2.2\%) seropositive patients, there were 3 (75.0\%) from the rural area, and 1 (25.0\%) from the urban area which was statistically significant \( (P = 0.001) \).

**Discussion**

Parasitic infections, especially helminthic diseases, have adapted to their host during long-term infections, which usually lead to chronic disease with reduced mortality. During this evolutionary adaptation to their hosts, including vertebrates, parasites have helped modulate several molecular and physiological mechanisms, for example, the host immune system. Thus, the parasitic helminths elicit a modulated Th2 responses in their vertebrate hosts, resulting in immune response with a well-controlled inflammatory component, including inhibition of pro-inflammatory cytokines and induction of hyporesponsive state by IL-10 and produces Treg cells [12]. In addition, the health hypothesis suggests that the absence of helminthic infections in the population of developed countries and the absence of parasitic immune stimulants increase autoimmune diseases with an exacerbated inflammatory component such as asthma and allergies [17]. Therefore, it can be concluded that infection with helminthic diseases such as *Fasciola hepatica*, from which various immunomodulatory molecules have been isolated [18], can lead to a milder course of COVID-19 disease. We speculate that *Fasciola hepatica*, can lead to decreased immune responses, thereby reducing the severity and symptoms of COVID-19 disease and, consequently, the hospitalization of COVID-19 patients. Therefore, it can be hypothesized that the prevalence of fascioliasis in COVID-19 patients who had admitted to the hospital is lower than the normal population.

In the current study, the frequency of *Fasciola hepatica* infection in COVID-19 patients was 2.2\%, significantly higher than the healthy control (without COVID-19 symptoms) (4.3\%). However, as the reported prevalence of fascioliasis in the general population in Iran up to 50.0\%, the relatively low frequency of *Fasciola* infection in our control group required cautious interpretation.

Of the 4 COVID-19 patients seropositive to *Fasciola*, 3 (75.0\%) were female. However, this was higher than the overall proportion of the female patients (47.3\%). Fascioliasis is seen more frequently among middle-aged adults probably due to exposure to the infectious agent. The relatively gradual increase in
seroprevalence associated with age suggested that the ingestion of metacercariae in water or leafy vegetables that grow near water, which is greatest during the adulthood years, may not be the principal mechanism by which persons are exposed to *Fasciola hepatica*.

In this study, COVID-19 consisted of various age groups from young adults to elderly, age ranging between 14 and 72 years. The seropositivity rate was not affected by age, though there were significantly more seropositive COVID-19 patients who were housekeeper.

A main finding of the study was the significant different between seroprevalence in the rural population compared with the urban population to *Fasciola*. Of the four patients classified as having anti-*Fasciola* antibodies, 75.0 percent were resided in the rural area. This observations suggested that population from rural area were more likely to be exposed to the parasite.

This study has some limitations. First, this study is a case-control study at a particular time, so the causal relationship between the research variables cannot be fully inferred. Second, because of the limited studies of *Fasciola* infection in patients with COVID-19, the results of this study cannot be consistent or inconsistent with other studies.

**Conclusion**

Fasciola infection is a helminthic diseases and preventable and treatable disease. Our findings in the present study further corroborate some observation of an association between Fasciola infection and COVID-19. Therefore, it is suggested by conducting more studies and achieving more reliable results regarding the effects of this parasites infection on the human immune system and their effect on the onset and symptoms of other diseases such as COVID-19, which have not yet been effectively.

**Abbreviations**

B cell: B lymphocytes; Breg cell: Regulatory B lymphocytes; C: Centigrade; COVID-19: Coronavirus disease 2019; CT scan: Computed tomography scan; ELISA: Enzyme-linked immunosorbent assay; IgG: Immunoglobulin G; *IL-10*: Interleukin-10; mL: Milliliter; OD: Optical density; PCR: Polymerase chain reaction; RPM: Revolutions per minute; SD: Standard deviation; SPSS: Statistical package for the social sciences; Th cell: T-helper lymphocytes; Treg cell: Regulatory T lymphocyte.

**Declarations**

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**Authors’ contributions**
FB, MZ, AH, HR, and FF worked on the data collection. FB, MZ wrote the manuscript following discussions with FF. HR did the statistical analysis. FB, MZ, and FF revised the paper and improved the technical quality of the manuscript. FB and MZ were the project coordinator and participated in all parts of the work. All authors approved the final version of the paper.

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**Availability of data and materials**

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

**Ethics approval and consent to participate**

All experimental protocols were approved by a named institutional/licensing committee. Specifically, human blood collection and serological experiments (and relevant protocols) were approved by the Ethics Clearance Committee of the Alborz University of Medical Sciences (ECCABZUMS) (IR.ABZUMS.REC.1399.215). Informed consent was obtained from all subjects, and all methods were carried out in accordance with the relevant guidelines and regulations of ECCABZUMS. So that, written informed consent was obtained from the patients' guardians on behalf of the children or minors and the illiterate participants enrolled in this study.

**Consent for publication**

Not applicable.

**Competing interests**

The authors have no conflicts of interest to disclose.

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