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

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Research Article

Keywords: Seroepidemiology, Fascioliasis, Fasciola hepatica, Coronavirus, COVID-19, ELISA

Posted Date: March 4th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-257860/v1

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 (ß), 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-*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 *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-*Fasciola hepatica* antibodies, as compared to 9 (4.3%) among healthy group. This was statistically significant ($P = 0.041$) as shown in Table 1.

Table 1

Seroprevalence of antibodies to *Fasciola hepatica* in the coronaviruses[a] patients and control group

	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 OVID-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

Acknowledgements

The authors would like to thank all staff of the Department of Parasitology of Alborz University of Medical Sciences, Iran.

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.

Funding

This study was supported by the Vice Chancellor for Research of Alborz University of Medical Sciences (Project No. 3877-22). The funding bodies had no role in the design of the study and collection, analysis, and interpretation of data and in writing the manuscript.

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/licencing 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.

References

1. Molyneux DH, Savioli L, Engels D. Neglected tropical diseases: progress towards addressing the chronic pandemic. Lancet. 2017;389:312-25.
2. Mas-Coma S, Bargues MD, Valero MA. Fascioliasis and other plant-borne trematode zoonoses. Int J Parasitol. 2005;35:1255-78.
3. Dietrich CF, Kabaalioglu A, Brunetti E, Richter J. Fasciolosis. Z Gastroenterol. 2015;53:285-90.
4. Haswell-Elkins MR, Elkins DB. Food-borne Trematodes, in: Cook, G.C. (ed.), Manson’s Tropical Diseases. London: Saunders; 1996. p. 1461-1464.

5. Mas-Coma MS, Esteban JG, Bargues MD. Epidemiology of human fascioliasis: a review and proposed new classification. Bull World Health Organ. 1999;77:340.
6. Mas-Coma S. Human fascioliasis: epidemiological patterns in human endemic areas of South America, Africa and Asia. Southeast Asian J Trop Med Public Health. 2004;35:1-11.
7. Mitra AK, Mawson AR. Neglected tropical diseases: epidemiology and global burden. Trop Med Infect Dis. 2017;2:36.
8. Moghaddam AS, Massoud J, Mahmoodi M, Mahvi AH, Periago MV, Artigas P, Fuentes MV, Bargues MD, Mas-Coma S. Human and animal fascioliasis in Mazandaran province, northern Iran. Parasitol Res. 2004;94:61-9.
9. Ashrafi K. The status of human and animal fascioliasis in Iran: A narrative review article. Iran J Parasitol. 2015;10:306-28.
10. Rokni MB, Massoud J, O'Neill SM, Parkinson M, Dalton JP. Diagnosis of human fasciolosis in the Gilan province of Northern Iran: application of cathepsin L-ELISA. Diagn Microbiol Infect Dis. 2002;44:175-9.
11. Rahimi MT, Ashrafi K, Koosha S, Abdi J, Rokni MB. Evaluation of Fast-ELISA versus standard-ELISA to diagnose human fasciolosis. Arch Iran Med. 2011;14:18-21.
12. Maizels RM, McSorley HJ. Regulation of the host immune system by helminth parasites. J Allergy Clin Immunol. 2016;138:666-75.
13. Li H, Liu SM, Yu XH, Tang SL, Tang CK. Coronavirus disease 2019 (COVID-19): current status and future perspectives. Int J Antimicrob Agents. 2020;55:105951.
14. Ahmad T, Khan M, Haroon, Musa TH, Nasir S, Hui J, Bonilla-Aldana DK, Rodriguez-Morales AJ. COVID-19: Zoonotic aspects. Travel Med Infect Dis. 2020;36:101607.
15. Hatami H, Asmar M, Masoud J, Mansouri F, Namdaritabar H, Ramazankhani A. 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;3:266-72.
16. Sarkari B, Ghobakhloo N, Moshfea A, Eilami O. Seroprevalence of human fasciolosis in a new-emerging focus of fasciolosis in Yasuj district, southwest of Iran. Iran J Parasitol. 2012;7:15-20.
17. Jackson JA, Friberg IM, Little S, Bradley JE. Review series on helminths, immune modulation and the hygiene hypothesis: immunity against helminths and immunological phenomena in modern human populations: coevolutionary legacies? Immunology. 2009;126:18-27.
18. Robinson MW, Donnelly S, Dalton JP. Helminth defence molecules-immunomodulators designed by parasites! Front Microbiol. 2013;4:296.

Figures

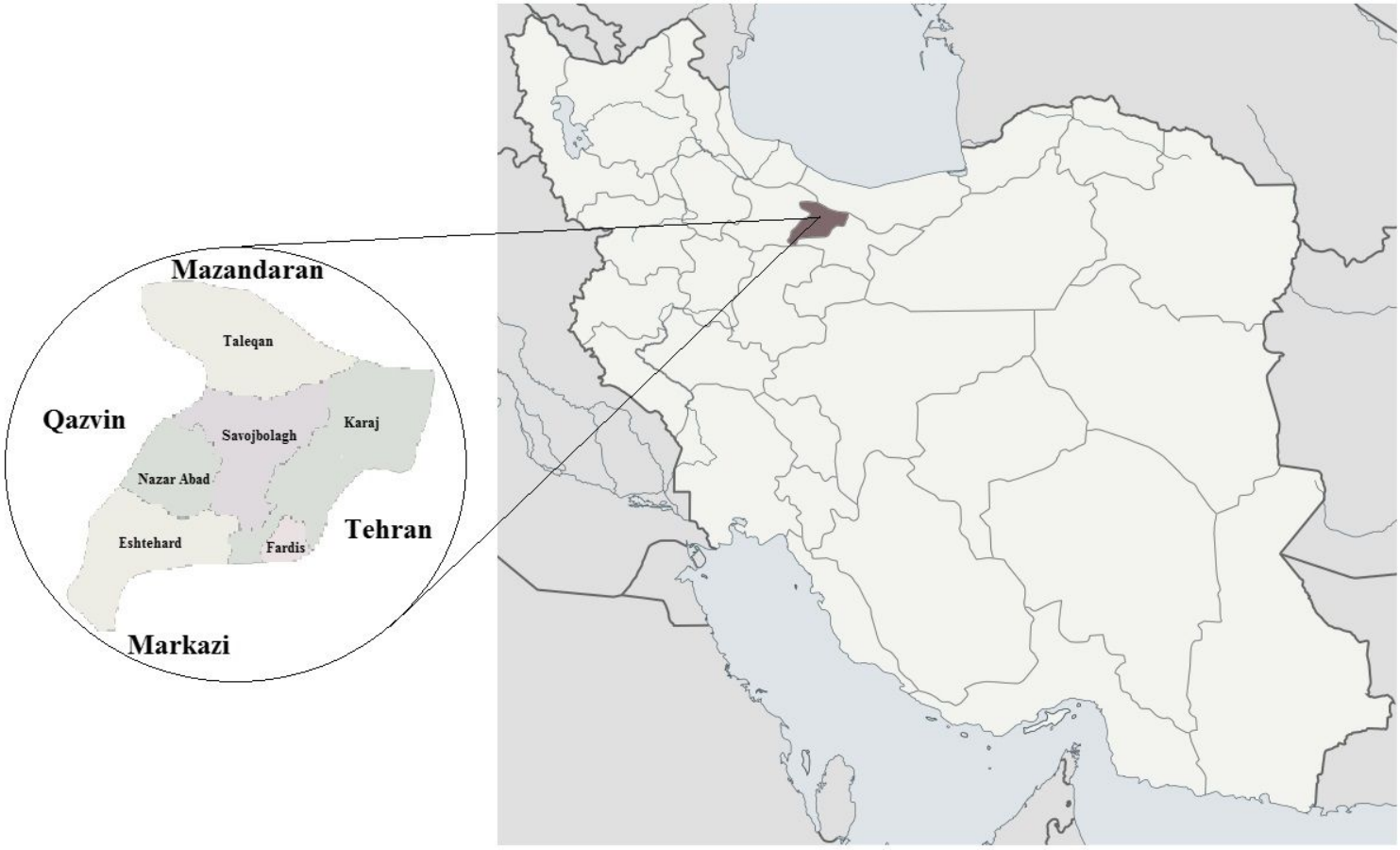

Figure 1

Regions in Alborz Province from where the serum samples were examined for Fasciola hepatica seropositivity. Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.