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Association between toxoplasmosis and COVID-19 infection: A cross-sectional study

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Abstract

Background: COVID-19 is a respiratory disease pandemic and a major global health problem that can cause acute respiratory distress syndrome (ARDS). Previous studies have shown an inverse link between toxoplasmosis and COVID-19. This study aimed to evaluate the association between COVID-19 and toxoplasmosis infection.

Methods: In this cross-sectional descriptive study, samples were taken from 360 patients, 50% of whom were men and 50% were women. Of the patients, 180 were determined to be COVID-19-positive by the ELISA kit, and 180 were in the control group. Some demographic characteristics, such as sex, age range, and occupation, were also recorded.

Results: This study was conducted on 180 COVID-19-positive patients, of whom 26.7% were in the 16-30-year age group, 25.1% were self-employed, and 31.7% had anti-*Toxoplasma gondii* antibodies. Among the 180 control patients, 21.1% had antibodies for *T. gondii*. Of the 57 patients who were co-infected with both COVID-19 and toxoplasmosis, men had higher infection rates (63.2%) than women (36.8%). There was a significant relationship between co-infections with COVID-19 and toxoplasmosis with occupation, but not with age.

Conclusion: The study found that people with toxoplasmosis infection have a 1.73 times higher risk of contracting COVID-19. The findings suggest that infectious agents could be a predisposing factor, possibly due to changes in cytokine levels.

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Introduction

In December 2019, the COVID-19 virus initiated a pandemic of respiratory disease that affected the lower respiratory tract and caused acute respiratory distress syndrome (ARDS). The disease was a global health emergency and was predominantly transmitted from person to person through respiratory droplets, contact, and secretions (1). The common symptoms of COVID-19 include fever, chills, cough, myalgia, shortness of breath, and headache. However, in severe cases, the disease can manifest as respiratory distress, acute kidney or liver failure, metabolic acidosis, coagulation disorders, and septic shock (1, 2).

Individuals with immunodeficiency, autoimmune disorders, and organ transplant recipients are at a high risk for COVID-19 infection. Studies have revealed an inverse relationship between toxoplasmosis and COVID-19, highlighting the significance of their association (3-8).

Toxoplasma is an intracellular protozoan that infects humans, birds, and mammals (9-11). Humans contract toxoplasmosis by eating raw or undercooked meat products containing tissue cysts or oocysts through contaminated food or by contact with infected cat feces. The prevalence of toxoplasmosis varies depending on factors such as health level, temperature, humidity, pet contact, and contact with soil (12-16). Most patients with a normal immune system remain asymptomatic, but 20-30% of cases show mild fever, lymphadenopathy, and skin rash. In congenital toxoplasmosis, patients with an immunocompromised state may develop severe manifestations such as central nervous system (CNS) disorders, encephalopathy, pneumonia, myocarditis, and brain calcification (12, 14, 17).

The level of community infection depends on many factors, such as health level, ambient temperature, humidity, contact with soil and pets, and the most common mode of transmission is through contact with infected cat feces (18). This opportunistic parasite is present in about one-third of the world's population. In Iran, some areas have reported a seroprevalence rate of more than 70%. Various methods are used to identify toxoplasmosis, including parasitology, histology, and various serological methods, such as the Sabin-Feldman dye test (DT), indirect hemagglutination (IHA), immunofluorescence assay (IFA), enzyme-linked immunosorbent assay (ELISA), and direct agglutination (DA). DNA-based methods, such as polymerase chain reaction (PCR) and real-time PCR, are also used for diagnosis (19, 20). This present study aimed to evaluate the seroprevalence of COVID-19 in individuals with toxoplasmosis infection and to establish the relationship between the two diseases.

Methods

The current study involved a cross-sectional descriptive analysis that included 360 patients referred to health centers in Saveh, Iran. Among the individuals, 50% were men and 50% were women. Blood samples were collected, and an ELISA kit provided by Pishtaz Teb, Iran, was used to classify 180 participants as COVID-positive, designating them as the experimental group. The remaining 180 participants tested negative and served as the control group. Demographic data, including sex, age range, and occupation, were also recorded through questionnaires. Finally, a comparison was made between the experimental group with anti-COVID-19 antibodies and an equal number of healthy individuals in the control group, with sex matching.

The study involved assessing the titers of IgG and IgM antibodies specific to anti-COVID-19 using the protocol provided by the kit manufacturer (Pishtaz Teb, Iran; SARS-COV-2 IgG & SARS-COV-2 IgM). Individual serum samples (100 µL) were transferred to 96-well plates coated with SARS-COV-2-specific antigens. The plates were then incubated for 30 minutes at 37 °C and washed 5 times using an ELISA washer. A conjugated enzyme (100 µL) was then added to each well and incubated for another 30 minutes before emptying and washing the contents 3 times. The next step involved adding 100 µL of the dye solution and keeping the plates in the dark for 15 minutes at room temperature. Afterward, 100 µL of the stop solution was added to complete the reaction. Finally, the plates' light absorption was read against the blank (100 µL of the dye solution, incubated in the dark for 15 minutes at room temperature, and then 100 µL of the stop solution) using an ELISA reader at a wavelength of 450 nm. A value above 1.1 was considered positive, while a value below 0.9 was deemed negative as per the manufacturer's instructions. The Pishtaz Teb kit had a sensitivity of 94.1 and 79.4 and specificity values of 98.3 and 99.3 for IgG and IgM antibody diagnostic tests for anti-COVID-19, respectively.

The present study utilized the Pishtaz Teb Company kit to measure the anti-*Toxoplasma* IgG antibody titer through the ELISA method. The kit utilizes the capture antibody approach, wherein the plate wells are coated with antihuman IgM and IgG antibodies. During testing, diluted serum samples are poured into the wells, where their IgG antibodies, including anti-*Toxoplasma* IgG, bind to the antihuman antibodies coated in the wells. After an initial washing step, the unbound antibodies are removed. Next, the *Toxoplasma* antigen is conjugated to Horseradish Peroxidase (HRP), and this antigen also binds to *Toxoplasma*-specific IgG antibodies in the serum samples and forms a complex. After another washing step, the dye solution is added to the wells, and the intensity of the resulting blue color is proportional to the immune complexes formed within the

wells. The addition of the stop solution turns the blue solution into yellow, which has the best light absorption at 450 nm. Finally, the kit defines values above 1.1 as positive and values below 0.9 as negative.

Chi-square and Fisher's exact tests were utilized for data analysis, and a confidence level of 95% was set. The significance level was considered less than 0.05.

Results

Out of the 180 people with COVID-19, 57 individuals (31.7%) had toxoplasmosis antibodies. Additionally, among the 180 people in the control group who did not have COVID-19 antibodies, 38 individuals (21%) tested positive for toxoplasmosis antibodies. Statistically, no significant difference was observed between the 2 groups, although the rate of toxoplasmosis cases was higher in the COVID-19 group, such that the odds ratio was 1.73; this means that the probability of infection with COVID-19 in individuals with toxoplasmosis infection is 1.73 times higher than in people without toxoplasmosis antibodies.

Based on the age distribution of people who had COVID-19 and toxoplasmosis in the studied groups, the percentages of individuals aged 0-15, 16-30, 31-45, 46-60, and over 60 years old were 6%, 7%, 9%, 3%, and 6%, respectively. Moreover, as can be seen in Table 1, the results of the occupation survey of individuals who had both COVID-19 antibodies and toxoplasmosis indicated that the highest infection rate was observed among the self-employed group.

Table 1. Frequency of co-infection with COVID-19 and toxoplasmosis based on the individuals' occupations

Occupation	Number	Percent (%)
Self-employed	45	25.0
Retired	6	2.8
Homemaker	39	21.7
Therapeutic	12	6.7
Driver	6	3.3
Employee	38	21.1
Student	34	18.9
Total	180	100

Discussion

Even with the frequent use of wide-ranging antimicrobial treatments among patients with respiratory infections related to the coronavirus, cases of co-infection with bacteria and fungi have been observed (21). COVID-19 patients often suffer from co-infection with Gram-negative bacilli and *Candida*. The virus can damage airway epithelial tissue, reduce mucosal clearance, and weaken immunity, leading to such co-infections (22). There have been reports indicating a negative correlation between COVID-19 incidence and certain parasitic diseases, such as malaria (where countries with endemic malaria were less likely to have cases of COVID-19), and the modulatory role of some parasites in the immune system based on some studies (23). However, other studies have shown conflicting results, indicating an increased risk of COVID-19 when a parasitic disease is present (24).

An infection with toxoplasmosis has been linked to higher severity of COVID-19 infection. This may be due to increased expression of *Toxoplasma*-associated PD-1 (programmed cell death protein 1) in lymphocytes, which is a critical checkpoint for T cell exhaustion. While it is a vital molecule for maintaining environmental tolerance, T cells become less effective when its expression is increased, leading to inhibitory signals that interfere with T cell proliferation, function, metabolic activity, and hemostatic self-renewal. These conditions, which compromise the first defense against the virus, are responsible for the considerable increase in the viral load, leading to severe clinical manifestations and potentially fatal outcomes (25-27).

An elevated level of serum anti-*T. gondii* IgG – which is also raised in COVID-19 patients – has been found to be even higher in cases classified as severe. This observation suggests that chronic toxoplasmosis reactivates more readily in COVID-19 patients due to the immune fatigue induced by the disease (24). This reactivation of toxoplasmosis exacerbates the intensity of COVID-19 and can be attributed to the significant increase in all cytokines, including IL-1β, IL-6, TNFα, IFN-γ, and IL-1α, which were the highest in severe COVID-19 cases among those infected with *T. gondii* (24). Serum cytokine levels play a crucial role in predicting the prognosis of COVID-19 and the likelihood of a cytokine storm. For instance, elevated levels of interleukin-6 in certain COVID-19 patients indicate a poor prognosis and may be suppressed with drugs such as tocilizumab (28). In this study, there was no significant relationship between COVID-19 and toxoplasmosis, but our results showed that the incidence rate in people who had toxoplasmosis antibodies was 1.73 times higher than those who did not have toxoplasmosis antibodies.

Experimental evidence indicates that cats and dogs can contract SARS-CoV-2 infections with or without displaying symptoms (29, 30). A recent study carried out in Spain revealed that stray cats were exposed to SARS-CoV-2 and had co-infections with other pathogens, including *T. gondii*. This suggests that animals with weakened immune systems may be more susceptible to SARS-CoV-2 infection (31). Latent toxoplasmosis, in general, appears to make human hosts more prone to experiencing various adverse effects from genetic factors and pathogens. In Minnesota, the USA, a study found that 8% and 1% of 239 domestic cats and 510 domestic dogs, respectively, had SARS-CoV-2 seroprevalence. Additionally, a study conducted in Germany reported a seroprevalence of 0.7% in cats during the first wave of COVID-19, which increased to 1.4% after the second wave (32). Latent toxoplasmosis, in general, appears to make human hosts more prone to experiencing numerous adverse effects from genetic factors and pathogens (33).

Meta-regression techniques used to analyze infection mortality rates based on age indicate an exponential correlation between age and COVID-19 infection mortality rates. The risk of mortality due to COVID-19 infection is believed to be very low in children and young adults but gradually increases with age. However, the results of the chi-square test performed in this study showed no correlation between age, occupation, and COVID-19 (34). Conversely, this analysis revealed a significant association between COVID-19 and sex, although other studies have reported an equal prevalence of the disease among both sexes. Regardless of age, men who contract COVID-19 face a higher risk of severe outcomes and death.

Conclusion

According to the study's findings, individuals who have a toxoplasmosis infection are more likely to contract COVID-19. There was no significant association found between age, occupation, and COVID-19 infection. The prevalence and activity of *T. gondii* were observed to be higher in severe cases of COVID-19 and were linked to increased levels of poorly prognostic cytokines in the bloodstream. Moreover, sex displayed a significant correlation with COVID-19 infection. Together, the outcomes of this and comparable studies propose that infectious agents may act as a predisposing factor, potentially due to changes in serum cytokine levels. Further investigation into cytokines and other factors is necessary to acquire a more comprehensive understanding of these associations.

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Ethical statement

This study has ethical approval from the Ethics Committee of Saveh University of Medical Sciences (Code: IR.SAVEHUMS.REC.1399.035). Informed consent was obtained from all the participants. All the procedures were performed in accordance with relevant guidelines and regulations.
All the experiments were carried out in accordance with the guidelines of the Ethics Committee (R.SAVEHUMS.REC.1399.035) of Saveh University of Medical Sciences, Saveh, Iran.

Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author contributions

Seyedmousa Motavallihaghi, Nastan Barati, and Eissa Soleymani: Conceptualization, Methodology, Data analysis, Writing, and Editing. Ali Ehsan Shahbazi, Behjat Ranjouri, and Abolfazl Khandan Del: Investigation, Data collection, Data analysis, and Writing (Original Draft). Nemat Azizi, Pegah Khandan Del, and Mehran Bakhtiari: Methodology and Writing.

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