Archives of Razi Institute, Vol. 78, No. 2 (2023) 675-680

DOI: 10.22092/ARI.2022.359481.2432

Original Article

Serological Detection for Toxoplasmosis among Patients with Covid-19 in Thi-Qar Province

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Received 23 July 2022; Accepted 13 August 2022
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Abstract

Covid-19 is a viral disease that affects humans caused by a type of virus belonging to the family Coronaviridae called the SARS-CoV-2 virus. The parasitic infection associated with this disease affects the host's immune response *regulation.* The levels of IgG and IgM of *Toxoplasma gondii* in the serum of patients with COVID-19 were measured by immunoassay of the patient's sera by ELISA. Also, the level of interferon-gamma (IFN-γ) in a covid-19 patient with or without Toxoplasmosis was evaluated. 120 samples were collected, 60 were positive for COVID-19, confirmed by clinically and radiographic examination, and 30 were in the control group. The results showed a significant difference between the infection with Covid-19 and *T. gondii* during the chronic phase of Toxoplasmosis compared to the negative relationship in the acute phase. The results of INF-γ levels among Covid-19 patients were positive for all samples included in the test (30 Covid-19 patients and 30 patients COVID-19(+)/*T. gondii* IgG) compared to the control group. The chronic form of Toxoplasma disease, due to change in the production of this interferon, the COVID-19 infection has changed.

Keywords: *Toxoplasma gondii*; interferon gamma; immune response; COVID-19

1. Introduction

Coronaviruses have been a constant pandemic since 2003. Humanity has been in a health security emergency related to the coronavirus (1). COVID-19 has been linked to asymptomatic or symptomatic variants of the virus and acute viral pneumonia with respiratory failure, multi-organ, systemic imbalances, sepsis, septic shock, and death. Since its breakout, COVID-19 has been linked to a multi-organ effect (2). Many other micro-organisms have been associated with coinfection with covid-19 like parasites, bacteria, viruses, and fungi (2). Chronic parasite infections have been found to affect the clinical symptom of another disease, which is associated with the effect on the immune system and the direct effect on the regulation of the host's immune responses. Chronic parasite infection can have both positive and negative consequences. COVID-19 has been found to have an inverse connection with intestinal worms, schistosomiasis, and malaria infection in recent studies (3). *Toxoplasma gondii* is a globally crucial intracellular protozoan parasite that can infect, survive, and multiply nearly all mammalian cells. *T. gondii* is still a neglected parasitic ailment 110 years after its discovery (4). Certain common illnesses, such as Toxoplasmosis, combined with a less sanitary lifestyle may activate the human immune system, allowing for some protection against CoVID-19, according to the so-called "hygiene theory" (5). Interferon-gamma (IFN-γ) mediated immune responses are required for regulating tachyzoite proliferation during both acute

acquired infection and reactivation of infection in the brain (6). Interferon-gamma (IFN- γ) is a well-known antiviral cytokine that plays a crucial role in viral replication (7). In this study, by examining the immune response in patients with CoVID-19 and people with chronic Toxoplasmosis, the changes in IFN-γ immune factors were evaluated.

2. Materials and Methods

2.1. Experimental Design and Sample Collection

The total number of patients with COVID-19 included in the present study was 60 who were referred AL-Hussein teaching hospital in Nasiriyah city, Thi-Qar province, during the period extended from March 2022 to June 2022. The patients were examined clinically and diagnostically by Chest X-ray, COVID-19 IgG, and COVID-19 IgM. 30 healthy people were also included in the current study as a control group. Age, Sex, Location, and occupation of all 90 people (60 patients and 30 controls) included in the following study were checked based on a designed questionnaire.

2.2. Detection of *T. gondii and* COVID-19 Antibodies

Five ml of blood was taken from each individual and was drawn by vein-puncture using 5 ml disposable plastic syringes under sterile conditions. Blood was collected in the gel tube and left until clotted at room temperature for one hour. After blood clotting, it was centrifuged at 4000 rpm for 10 minutes, and then the serum was divided into two equal parts in Eppendorf tubes for immunological tests (COVID-19 IgG, COVID-19 IgM, *Toxoplasma IgG*, *Toxoplasma IgM*, INF-Y), then stored in -20◦C. Each part of sera was used once to avoid repeated thawing and freezing. All materials (i.e., reagents and sera) were allowed to stand at room temperature before use (8, 9).

The *Toxoplasma* IgG/IgM EIA Test and IFN-γ Kits is a solid phase enzyme immunoassay based on an indirect principle for the qualitative and quantitative detection of IgG/IgM antibodies to *T. gondii* in serum or plasma.

2.3. Statistical Analysis

The statistical analysis tested the samples using a continuous variable as means and SDs, medians, interquartile ranges for categorical variables, and frequency rates and percentages for categorical variables (IQRs). Using the $\chi 2$ test, proportions for categorical variables were compared by SPSS. The analyses considered a p-value of 0.05 ($P<0.05$) statistically significant.

3. Results

The current study targets patients with Covid-19 to explain the presence of *Toxoplasma gondii* Co-infection associated with Covid-19 and its relation with the immune response to IFN-γ. The results showed significant differences between the infection with Covid-19 and *T. gondii* during the chronic phase of Toxoplasmosis, where the patients with Covid-19 revealed (53.3 %) infection *T. gondii* after measuring the titer of IgG for *T. gondii* in comparison with (46.7 %) of patients were negative for Toxoplasmosis. Statistical analysis was shown in table 1 to explain the relationship between the infection with Covid-19 and the chronic phase of *T. gondii* ($P<0.001$).

Table 1. Prevalence rate of COVID-19 infection with *T. gondii (IgG)*

Toxo IgG	Case	No. (%)	Mean
Patient	Positive	32 (53.3)	218.4±206.6
	Negative	28 (46.7)	8.69±0.91
Control	Negative	30 (100)	5.19±3.82

The study of the relationship between infection with covid-19 and acute phase toxoplasmosis showed no significant relationship between patients with covid-19 and those with the acute phase of Toxoplasmosis. The difference between infection with covid-19 and coinfection with *T .gondii* in the acute stage is shown in table 2.

Table 2. Percentage of the prevalence of covid 19 in acute toxoplasma (IgM) patients

Toxo IgM	Case	No. (%)	Mean
COVID-19 Patient	Positive	2 (3.3)	1.32±0.19
	Negative	58 (96.7)	0.11±0.1
Control	Negative	30 (100)	0.27±0.25

The age of Covid-19 patients with infection with *T. gondii* (IgG, IgM) was also checked based on the requirement of the present study, and the statistical analysis did not show significant differences, as explained in a table 3. The high rate of infection with *T. gondii* (IgG) was reported among Covid-19 patients in (41-50) age groups, while the low rate of infection with *T. gondii* (IgG) was reported among (10-20) age groups of Covid -19 patients. *T. gondii* (IgM) infection was not reported among Covid-19 patients. Whom have (10-20, 41-50, 51-60, 61-70) age groups while the Covid-19 patients whom have infection with *T. gondii* (IgM) were (21-30 and 31-40) age groups where reporting (1.7%) rate of infection (*P*-value=0.76).

Table 3. Effect of Age on the prevalence of COVID-19 patients with *Toxoplasma gondii* (IgG and IgM)

Age (year)	No. (%) (IgG)	Mean	No. (%) (IgM)
10-20	2 (3.3%)	321	0
21-30	7 (11.7%)	207.5	1 (1.7%)
31-40	7 (11.7%)	164	1 (1.7%)
41-50	8 (13.3%)	216.3	0
51-60	3 (5.0%)	160.5	0
61-70	4 (6.7%)	210.3	0

The effect of sex of patients with Covid-19 infection with *T. gondii* (IgG, IgM), as in table 4, was not show significant differences.

Table 4. Effect of sex on prevalence of COVID-19 patients with *T. gondii (*IgG and IgM)

Sex	No. *T. gondii* (IgG) (%)	Mean	No. *T. gondii* (IgM) (%)
Male	13 (21.7)	212.6±200	1(1.7)
Female	18 (30)	218.5±206	1(1.7)

Statistical analysis showed a no-significant difference in the geographic distribution of Covid-19 patients and infection with *T. gondii* (IgG, IgM). Covid-19 patients living in urban cities consist (31.7%) of infection with *T. gondii*, while the infection rate with *T. gondii* among Covid-19 patients that lived in rural cities was 20 % (Table 5). *P*-value = 0.53.

Table 5. Geographic distribution of COVID-19 patients with *T. gondii (*IgG and IgM)

Location	No.(%) (*T. gondiii* IgG)	Mean	No.(%) (*T. gondiii* IgM)	Mean
Urban Covid-19 patients	19(31.7)	218.4±206.6	2(3.3)	1.25±0.12
Rural Covid-19 patients	12(20)	206±193.9	zero	

The results of INF-Y levels among Covid-19 patients included in the present study were positive for all samples included in the test (30 Covid-19 patients and 30 patients COVID-19(+)/*T. gondii* IgG) compared to the control group. Statistical analysis (χ^2=4368.52) showed significant differences ($P \leq 0.05$) between Covid-19 patients and COVID-19(+)/ *T. gondii* IgG compared with the control group (Table 6). *P*-value=0.003.

Table 6. Level of Interferon-gamma in COVID-19 patients and COVID-19+*Toxoplasma gondii* (IgG)

INF-Y	No. (%)	Mean
COVID-19(+)	30(50)	46.1±39.7
COVID-19(+)/*T. gondii* IgG(+)	30(50)	2289±2283
Control	28(100)	8.29±2.8

4. Discussion

There have been approximately 25 million Covid-19 infections worldwide as of September 2020, with over 800,000 individuals dying due to the illness. Scholars are increasingly recognizing that insights from the social and behavioral sciences play a crucial role in restricting the spread of the virus, particularly regarding the spread of disinformation regarding the infection (8). Covid-19 became the third largest cause of mortality in the United States, lowering life expectancy significantly for that year. Covid-19 claimed more lives in the United States than in any other country, with one of the most significant mortality rates in the world (9). On February 14, 2020, Egypt reported the first case of COVID-19 in Africa, barely 14 days after WHO

declared the outbreak a public health emergency of worldwide concern. The Covid-19 spread to Africa in less than three months, and the reported cases show that the epidemic spreads much slower on the continent than elsewhere (10). Researchers confirmed the first case of COVID-19 in Iraq on February 24, 2020, at Al-Najaf city (11). Iraq's government has put several health restrictions on local and foreign travel. For example, travelers returning from the COVID-19 outbreak area are quarantined for at least 14 days and tested for the SARS-CoV-2 virus using a PCR test (12). Iraq has verified more than 234,934 cases of COVID-19 and more than 7042 deaths as of August 31, 2020 (13). This study targets covid-19 patients as a new pandemic disease related to *T. gondii* infection.

The current study showed a significant difference between the infection with Covid-19 and *T. gondii* during the chronic phase of Toxoplasmosis compared with the negative relationship in the acute phase. According to a study by Darweesh, Abdulrazzaq (14), anti-*T .gondii* antibodies (IgG) were found in the serum of COVID-19 patients using a commercial enzyme-linked immunosorbent assay kit, indicating that latent *Toxoplasma* infection is common among COVID-19 patients. Parasite coinfection is linked to a lower incidence of severe COVID-19 in African patients (15). Interferon-gamma (IFN-γ) is a cytokine produced by T helper 1 (Th1) cells that inhibits the growth and survival of intracellular pathogens. IFN-γ stimulates the gene expression of several effector molecules in innate immune cells such as macrophages and dendritic cells (16). Patients with ocular hypertension who produce high intraocular IFN-γ levels after toxoplasmic retinochoroiditis reactivation can be recognized as high IFN-γ releasers in cytokine release assays by peripheral blood mononuclear cells (PBMCs) (17). When co-cultured with human monocytes, the *T. gondii* effector *T. gondii* dense granule protein (GRA15) plays an essential role in suppressing IFN-inducible indole-2,3-dioxygenase 1(IDO1)-dependent anti-*T. gondii* responses in human brain and liver cells (18). SARS-CoV2 reactive IFN-γ CD8+ T cells were found in a non-negligible percentage of individuals with moderate to severe COVID-19 (19). Reduced circulating IFN-γ is a risk factor for lung fibrosis in COVID-19 patients (20), while another study found a significant limitation of interferon-gamma release assay (IGRA) testing in severely ill COVID-19 patients. These data demonstrate a focused Th2 immune response with inhibition of IFN-γ signaling (21).

Furthermore, another study found that high levels of IFN-γ in moderate cases compared to low levels in extreme cases resulted in significant modification of the human immune response to COVID 19 infection by parasite infections (22). Acute Plasmodium infection can protect against *Ebola Virus* by producing protective IFN-γ. These findings have implications for anti-malaria medicines used in Africa during recurrent *Ebola Virus* outbreaks (23), while another study found that IFN-γ reduced varicella-zoster virus replication and early IE62 protein-mediated transactivation in a cell line-dependent way. JAK/STAT1 signaling inhibits *varicella-zoster virus* in response to IFN-γ (7). Another study found that interferon gamma-inducible protein 16(IFI16) may detect HBV DNA during infection. Early alterations in IFI16 mRNA can help predict HBeAg seroconversion during interferon therapy (24).

The results showed a significant difference between the infection with Covid-19 and *T. gondii* in the chronic and the acute phase. The results of INF-γ levels among Covid-19 patients were positive for all samples included in the test (30 Covid-19 patients and 30 patients COVID-19(+)/*T. gondii* IgG) compared to the control group. Statistical analysis (χ^2 = 4368.52) showed significant differences ($P \leq 0.05$) between Covid-19 patients, COVID-19(+)/*T. gondii* IgG compared with the control group. Interferon-gamma (γ-IFN) is produced by activated T-lymphocytes and natural killer cells (more than it is activated by viral infections, this interferon is involved in the activation of NK1 (macrophages), antiviral and antibacterial activities .The chronic form of Toxoplasma disease,

due to change in the production of this interferon, the COVID-19 infection has changed.

Authors' Contribution

Study concept and design: A. K. K.
Acquisition of data: A. A. A.
Analysis and interpretation of data: A. K. K.
Drafting of the manuscript: A. A. A.
Critical revision of the manuscript for important intellectual content: A. K. K.
Statistical analysis: A. A. A.
Administrative, technical, and material support: A. K. K.

Ethics

This study was approved by AL-Hussein teaching hospital of Iraq and written informed consent of penitents.

Conflict of Interest

The authors declare that they have no conflict of interest.

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