BENHA MEDICAL JOURNAL bmj

**Print ISSN** 1110-208X

**Online ISSN** 2357-0016

Original article

# Does Latent Toxoplasmosis Impacts SARS-CoV-2 Infection Severity and Outcome: A Cross-Sectional Study in Benha, Egypt

Asmaa A. Elkholy [a] , Ghada H. Omar [a] , Ayman M. Elbadawy [b], Rasha Omar [b], Amira Mohamady[b], Rabab E. Omar [a] , Amira E. Zaki [c] , Eman M. Araby [d] , Seham G Ameen [e], Ahmed ezzat [b]

[a] Department of parasitology, Benha faculty of medicine, Benha University, Egypt. [b] Department of internal medicine, Benha faculty of medicine, Benha University, Egypt . [c] Department of microbiology, Benha faculty of medicine, Benha University, Egypt. [d] Department of public health and community medicine, Benha faculty of medicine, Benha University, Egypt . [e] Department of clinical pathology, Benha faculty of medicine, Benha University, Egypt .

**Correspondence to:** Asmaa A. Elkholy, Department of parasitology, Benha faculty of medicine, Benha University, Egypt.

**Email:**

asmaakholy787@gmail.com

**Received**:14 November 2022

**Accepted**: 22 December 2022

## Abstract

**Background:** The unique single-stranded RNA virus COVID-19 is to blame for the initial viral pneumonia outbreak in China that quickly turned into a pandemic. The "immune exhaustion" which flares up viral multiplication and hasty clinical results, is one of the primary criteria of the immune response against COVID-19 and also, it is a common side effect of chronic infections with Toxoplasma gondii. The severity of COVID-19 could possibly be increased by the toxoplasmosis-associated immune depletion by making it worse**. Aim of study**: Our goal was to ascertain the prevalence of coinfection with latent toxoplasmosis in COVID 19 positive patients as well as its impact on the progression of the infection and the fate of the disease. **Patients &Methods**: 50 patients with PCR-confirmed COVID-19 infection were recruited for this cross sectional cohort prospective study at Benha university hospitals. They underwent Toxoplasma IgG testing by ELISA and had their conditions monitored to assess the COVID-19 infection's outcome. **Results:** Toxoplasma IgG seropositivity was found in 29(58%) patients while history of animal contact was 48.3% and 38.1% in both IgG positive and negative groups respectively. Toxoplasma Positive group showed higher incidence of: long hospital stay (20.7±7.3), ICU admission (72.4%), need for ventilation (55.3%), and more lung affection and sever CT findings

(24.1%) and higher death rate (10.3%). All these previous differences were statistically significant. **Conclusion**: The concurrent latent toxoplasmosis infection in (SARS-CoV-2) patients was linked to a more severe course and a worse result of COVID-19 infection.

**Keywords**: COVID-19; Parasitic infections; Toxoplasmosis

## Introduction:

The novel coronavirus SARS-CoV-2 (COVID-19), a strain of SARS-related coronavirus (SARSr-CoV), was first discovered in December 2019 in Wuhan, the capital of Hubei, China [1]. The dynamics of COVID-19 transmission are mostly influenced by respiratory droplets and close contact with infected individuals (carriers or sick) [2]. The rapid emergence of a pandemic and high infectivity of COVID-19 led the WHO to classify it as a severe infectious agent. Furthermore, it has the potential to result in fatal outcomes in a few days to a few weeks for susceptible individuals, including severe pneumonia , acute respiratory distress syndrome (ARDS) and even multi-organ dysfunction and death [3–5]. Type I interferon (IFN1), tumor necrosis factor (TNF), and other cytokines may have a significant collaborative role in the viral immune response, according on theoretical evidence about COVID-19 from earlier outbreaks of other coronaviruses [6]. Therefore, pulmonary histological abnormalities include bilateral diffuse alveolar destruction, cellular fibromyxoid exudates, pneumocyte desquamation, formation of the hyaline membrane indicative of ARDS, and interstitial infiltrate of mononuclear inflammatory cells, primarily lymphocytes, in both lungs [7]. Additionally, it has profound effects on every organ, causing edoema, vasoconstriction, inflammation, and hypercoagulability. There have been reports of myocardial infarction, ischemic stroke, and deep vein thrombosis, as well as embolism development and disseminated intravascular coagulation [8]. To ensure their survival inside their hosts, several parasites might modify the immune system [9&10] as trematodes, cestodes, and nematodes [11–15]. Th1 cells are suppressed by the type 2 responses that are induced. Additionally, the increased numbers of Th2 cells and alternatively activated macrophages influence the cytokine profiles to favour IL-4, IL-5, IL-9, and IL-13. Immunomodulatory

substances secreted by helminths alter IL-10 production as well as the growth of regulatory T (Treg) cells and regulatory B cells, which results in a greater suppression of type-1 responses [16–18]. Regarding this, earlier research has shown that helminthic infections may have therapeutic benefits in treating some autoimmune and allergic reactions [19 & 20].

The potential for helminthic co-infection to affect COVID-19 severity has recently been questioned by researchers [21]. Curiously, a 2018 study found that IL-4 response during helminth infections can promote antigen-specific CD8+ T cell effector responses in the lung that improve control of viral infection [22]. Similar to this, earlier research using animal models showed that parasites can protect against viral infections, or the "parasites versus virus phenomenon" [23]. The severity of viral infections was also seen to be modulated by protozoal infections. Along with the potential protective interaction between *Plasmodium spp* and the Chikungunya virus, concurrent *G. lamblia* infection lessens the severity of diarrheal episodes in rotaviruses [24 & 26].

Surprisingly, COVID-19 showed minimal improvement after receiving the standard anti-viral therapy. Additionally, the pathogenic setting of the SARS CoV2 Coronavirus seems to mimic several parasites. As an example, the virus attacks the heme group (porphyry) in the RBCs, releasing iron and depriving the body of oxygen with a sharp rise in cytokines, much like malaria [27].

The disease can eventually be eradicated once herd immunity has been established and the spread of the illness is restricted. Between vulnerable and infected participants, immune individuals serve as a buffer [28 & 29]. However, prior infections are necessary to build immunity. As a result, we wonder if endemic parasite illnesses and COVID-19 may be cross-reactive. We are unsure of the benefit of the non-hygiene hypothesis in this context. But prior studies found that the development of the immune system is influenced by exposure to a wide range of microbial antigens [30 & 31].

Regarding the health reports for rural areas in the USA, co-infection with *Ancylostoma duodenale* may worsen COVID-19 because it causes iron deficiency anaemia and drastically lowers iron reserves (ferritin) [32], aggravating the condition brought on by the iron release from SARS-CoV2. On the other hand, since both pathogens can generate a cytokine storm and coagulation state, co-infection with malaria may

aggravate the course of the illness brought on by either one [33].

The protozoan parasite *Toxoplasma gondii* infects about one-third of people worldwide. [34]. Since latent toxoplasmosis has long been thought to be asymptomatic, little effort has been made to find a medication that can both treat latent toxoplasmosis and eradicate the bradyzoites seen in cysts. But during the past 20 years, a number of studies have demonstrated that latent toxoplasmosis has serious negative impacts on the mental and physical health of infected individuals. Numerous illnesses, including fetal malformations, neonatal problems, and cardiovascular ailments have been discovered to have a substantial connection with toxoplasmosis. However, a significant positive connection was also seen with leukaemia, measles, and filariasis. A wide variety of observed relationships points to the effects of latent toxoplasmosis being quite general [35].

Additionally, it is well documented that Toxoplasma alters how the host's immune system functions, particularly by raising the levels of a few lymphokines, most notably IL-10 [36, 39], and altering the numbers of certain immunocytes [40].

It's interesting to notice that *T. gondii* and SARS-CoV-2 both use the same mechanism to activate innate immunity. In reality, the canonical pathway is used to activate toll-like receptors, such as TLR 2, TLR 4, and TLR 7, in both infections. However, it is also probable that some generated cytokines make COVID-19 more severe in toxoplasmosis patients [41, 42]. Thus, it is postulated that COVID-19 and *T. gondii* in hospitalised patients may be related.

**Methodology:**

The Benha University Faculty of Medicine's Research Ethics Committee gave its approval to this work. Informed consent was gained from patients or their guardians (RC 4-6-2021) depending on their condition after explaining the study's purpose to all participants.

***Subjects and study design:***

The present study was a cross-sectional prospective study. It was performed on 50 COVID-19 patients admitted to the Isolation Department of Benha University Hospitals with moderate or severe/critical manifestations. The duration of the study extended from October 2021 to January 2022.

***Inclusion and exclusion criteria:***

A throat swab PCR for COVID-19 was positive in every case. Even though there is a radiological and clinical suspicion. Comorbidities affecting COVID-19 outcome such as diabetes mellitus, chronic kidney disease, underlying cardiac condition, or chronic liver diseases as well as immunosuppressive conditions such as malignancy, immune mediated diseases, or immunosuppressive drugs will be excluded from the study besides, anti-*T. gondii* IgM seropositivity were excluded.

All patients underwent comprehensive history-taking, physical examinations, and clinical testing. They were categorized into four grades based on the results of CT scans as well as their oxygen needs. All patients also underwent *Toxoplasma* IgG testing, which allowed for the division of patients into groups with positive and negative results. ***Assessment of T. gondii seropositivity:***

According to the manufacturer's instructions, serum samples from each participant were tested for anti-*T. gondii* IgG antibodies using a commercially available ELISA kit, the Human Anti-*T. gondii* IgG kit (ab108776, Abcam, USA). To rule out acute infection, positive samples were tested for anti-*T. gondii* IgM antibodies using the Human Anti-*T. gondii* IgM Kit (ab108778, Abcam, USA). The amount of anti-*T. gondii* IgG antibodies was indicated in U/ml.

**Statistical analysis**:

Using IBM-SPSS Statistics for Windows, version 23.0, data were checked, coded by the researcher, and analyzed (Copyright IBM Corp., Armonk, N.Y., USA. 2015). Statistically descriptive data Calculations included means, standard deviations, medians, ranges, and percentages. Chi-square test was performed to assess the variation in frequency distribution between groups as a measure of significance. An independent t-test analysis was performed for continuous variables to compare the means of dichotomous data. *Toxoplasma* IgG levels and other characteristics in COVID-19 patients were correlated using Spearman's rho or the Pearson Correlation Coefficient, respectively. The sample size was not determined in a precise way. When it was 0.05 or below, a significant p-value was evaluated.

## Results:

Out of the 50 patients recruited in study 29 (58%) were *Toxoplasma* IgG antibodies positive and 21(42%) were IgG negative

while, anti-T. gondii IgM was not detected in any of the patients (Figure 1). The mean age of the positive group was 51.4±14.8 years most of them were females (55.2%), the urban residence was more common (93.1%), positive history of cat contact was 48.3%. regarding IgG negative group the mean age was 53.0±16.2 most of them were females with (38.1%) positive history of cat contact and the majority were from urban areas .there was no statistically significant difference between both groups regarding sociodemographic data (Table 1).

Regarding the laboratory findings in both Toxoplasma IgG antibodies positive and negative groups, no statistically significant difference was found when comparing both groups (Table 2).

In our study, we observed the occurrence of renal impairment and elevated liver enzymes, and bilirubin which were more elevated in the positive IgG group than in the negative group but the difference was statistically insignificant. However, inflammatory markers such as CRP, serum ferritin, and D-dimer in both groups were elevated but more in the negative group, and also the difference was statistically insignificant. (Table 2)

The seropositive group spent significantly longer periods in the hospital than the negative group. There was a statistically significant difference between the positive & negative groups considering their COVID infection outcome. ICU admission rate was higher among the positive group (72.4%) vs. (42.9%) in the negative group. Similarly, the death rate was higher among the positive group (10.3%) vs. (4.8%) in the negative group ($P<0.05$) (Table 3).

CT examination revealed a statistically significant difference between the positive & negative groups regarding chest findings. The positive group showed more lung affection rather than the negative group. The need for mechanical ventilation was significantly higher among the toxoplasmosis positive group (55.3%) vs. only (19.1%) for the negative group. (Table 4)

There was a statistically significant difference between *Toxoplasma* IgG positive & negative groups considering their COVID infection outcome. ICU admission rate was higher among the positive group (72.4%) *vs.* (42.9%) in the negative group. Similarly, the death rate was higher among the positive group (10.3%) *vs.* (4.8%) in the negative group. CT examination revealed a statistically significant difference between the positive & negative groups regarding

chest findings. The positive group showed more lung affection rather than the negative group. The need for mechanical ventilation was significantly higher among the toxoplasmosis positive group (55.3%) vs. only (19.1%) for the negative group (Table 4).

Correlation coefficient tests were performed to correlate IgG level with different variables including age, clinical data, and the disease outcome. It was positively correlated with CRP, ESR, and Creatinine which was statistically insignificant but a statistically significant positive correlation was observed with the RBCs count and COVID-19 outcome, other variables showed a statistically insignificant negative correlation (Table 5)

**Table (1):** Socio-demographic data of COVID-19 infection patients (seropositive vs. seronegative)

| **Variables** | **Positive N.=29** | **Negative N.=21** | **Test of significance** | **P-value** |
|---|---|---|---|---|
| **Age (years)** | 51.4±14.8 | 53.0±16.2 | ***t*** =.36 | .72 |
| **Sex** | | | | |
| Male | 13(44.8%) | 8(38.1%) | $X^2$ = .23 | .63 |
| Female | 16(55.2%) | 13(61.9%) | | |
| **Residence** | | | | |
| Urban | 27(93.1%) | 20(95.2%) | FET =1.7 | .43 |
| Rural | 2(6.9%) | 1(4.8%) | | |
| **Contact with animals** | | | | |
| Yes | 14(48.3%) | 8(38.1%) | $X^2$ =.51 | .47 |
| No | 15(51.7%) | 13(61.9%) | | |

**Table (2):** Laboratory findings of COVID-19 patients (seropositive vs. seronegative)

| **Variables** | **Positive N.=29** | **Negative N.=21** | **t-test** | **P-value** |
|---|---|---|---|---|
| **Hb%** | 11.4±1.8 | 10.4±2.1 | 1.7 | .09 |
| **RBCs (x $10^9$/uL)** | 6.2±10.2 | 6.1±8.1 | .04* | .97 |
| **WBCs (x $10^6$/uL)** | 9.4±4.3 | 11.3±6.6 | 1.2 | .26 |
| **Platelets (x $10^6$/uL)** | 187±78.5 | 215±94.7 | 1.1 | .27 |
| **Lymphocytes** | 1.2±.75 | 2.0±2.9 | 1.2 | .23 |
| **Neutrophils** | 7.7±4 | 15.3±29.9 | 1.2 | .26 |
| **Eosinophils** | .24±.24 | .32±.45 | .68 | .50 |
| **Basophils** | 1.6±7.7 | 0.19±.28 | 1.0 | .33 |
| **Monocytes** | .37±.32 | 1.8±5.5 | 1.2 | .24 |
| **Serum Ferritin (ng/mL)** | 495.5±366.7 | 489.5±368.2 | .06 | .96 |
| **D-Dimer** | 804.7±646.5 | 862.0±915.5 | .25 | .81 |
| **Urea (mg/dL)** | 66.3±36.9 | 50.1±30.9 | 1.7 | .09 |
| **Creatinine (mg/dL)** | 2.0±3.2 | 1.7±2.3 | .47 | .64 |
| **Uric acid (mg/dL)** | 7.8±9.3 | 8.9±13.9 | .33 | .74 |
| **AST (U/L)** | 40.2±25.8 | 37.5±21.0 | .39 | .69 |
| **ALT (U/L)** | 40.2±29.2 | 43.5±24.8 | .42 | .68 |
| **Bilirubin (mg/dL)** | 1.9±3.3 | 3.2±5.5 | .93 | .36 |
| **Albumin (g/dL)** | 16.1±15.6 | 16.8±16.5 | .15 | .88 |
| **CRP (mg/L)** | 62.5±59.9 | 69.0±76.9 | .27 | .79 |
| **ESR (mm/hr)** | 54.4±29.3 | 50.0±32.2 | .49 | .62 |

**Table (3)**: Length of Hospital Stay & outcome of COVID-19 infection (seropositive vs. seronegative)

| **Variables** | **Positive** | **Negative** | **Test of significance** | **P-value** |
|---|---|---|---|---|
| **Length of hospital stay (days)** | 20.7±7.3 | 15.6±9.4 | $X^2$ =2.05 | .038* |
| **Outcome** | | | | |
| Discharged | 5(17.2%) | 11(52.4%) | FET=6.69 | .029* |
| ICU | 21(72.4%) | 9(42.9%) | | |
| Died | 3(10.3%) | 1(4.8%) | | |

**Table (4):** CT findings & need for oxygen therapy of COVID-19 patients (Seropositive vs. Seronegative)

| **Variables** | **Positive** | **Negative** | **Test of significance** | **P-value** |
|---|---|---|---|---|
| **CT results** | | | | |
| No affection | 3(10.4%) | 5(23.8%) | FET=10.39 | .027* |
| Grade 1 | 5(17.2%) | 9(42.9%) | | |
| Grade 2 | 4(13.8%) | 1(4.8%) | | |
| Grade 3 | 10(34.5%) | 6(28.5%) | | |
| Grade 4 | 7(24.1%) | 0(0%) | | |
| **$O_2$ therapy** | | | | |
| No | 3(10.3%) | 5(23.8%) | FET=9.5 | .020* |
| Nasal prong | 5(17.2%) | 10(47.6%) | | |
| Air room | 5(17.2%) | 2(9.5%) | | |
| Ventilation | 16(55.3%) | 4(19.1%) | | |

**Table (5):** Correlation between toxoplasma IgG level & laboratory findings and outcome of COVID-19 infection

| IgG level<br>Variables | Pearson Correlation Coefficient | P-value |
|---|---|---|
| **Age** | -0.24 | 0.86 |
| **HB** | -0.53 | 0.72 |
| **RBCs** | 0.33 | **.02*** |
| **WBCs** | .012 | 0.94 |
| **Platelets** | -0.34 | 0.79 |
| **Lymphocytes** | -0.11 | 0.46 |
| **Neutrophils** | -0.04 | 0.76 |
| **Eosinophils** | 0.03 | 0.81 |
| **Basophils** | 0.25 | 0.07 |
| **Monocytes** | -0.09 | 0.55 |
| **Serum Ferritin** | -0.02 | 0.87 |
| **D-Dimer** | -.06 | 0.69 |
| **Urea** | 0.20 | 0.16 |
| **Creatinine** | 0.01 | 0.97 |
| **Uric acid** | -0.07 | 0.64 |
| **AST** | 0.17 | 0.23 |
| **ALT** | -0.06 | 0.69 |
| **Bilirubin** | -.04 | 0.77 |
| **Albumin** | -0.14 | 0.32 |
| **CRP** | 0.15 | 0.30 |
| **ESR** | 0.05 | 0.72 |
| **Length of hospital stay** | -0.08 | 0.59 |
| **Outcome** | **Spearman's rho = 0.52** | **0.000**** |

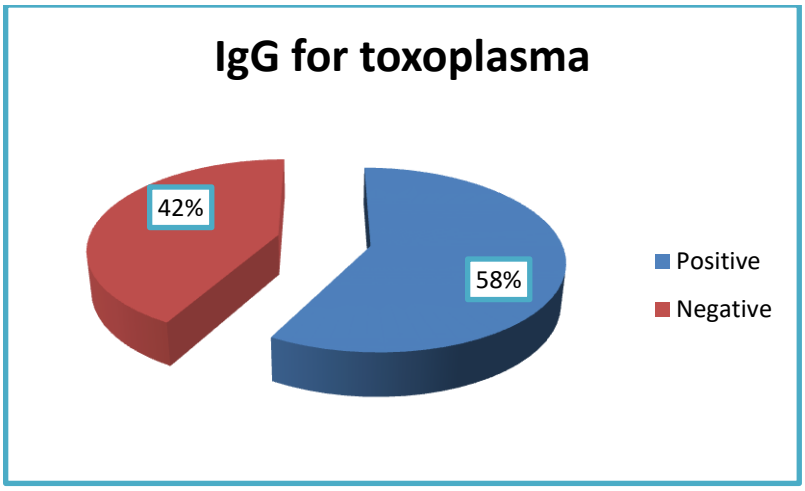


**Fig. (1):** Comparison between IgG levels among positive & negative groups for *Toxoplasma* infection in COVID-19 patients admitted to BUH

## Discussion

Our study was designed as a trial to understand the effect of T. gondii co-infection on the severity of COVID-19 manifestations, and due to the impact of widely distributed protozoal infection on the immune system. We tried to ascertain whether *T. gondii* can be considered a risk factor for COVID-19 severity. The fact that it is so common to explain both how it affects the severity of COVID-19 and how hazardous this illness is. Since mild COVID-19 cases were separated at their residences in accordance with Ministry of Health practice, only moderate and severe cases were included in the study population. The presence of specific IgG with the absence of IgM was used to diagnose the chronicity of toxoplasmosis [43].

In our study, the mean age of included patients was 51.4±14.8 years in IgG positive group and 53.0±16.2 years in IgG negative group and this increased incidence of severe manifestations with increased age can be explained by the association between aging of the immune system and increased liability to malfunction and apoptosis of immune cells with subsequent increase in growth and multiplication of the virus.

In our study, we found that in both groups no hematological indices were statistically different and so we could not correlate it with the outcome also this was in agreement with Moledina *et al*. [44] who reported that no hematological marker

was statistically significant at predicting inpatient mortality.

In our study, we found that lymphopenia was evident in both groups but more severe in *Toxoplasma* positive group. However, it was statistically insignificant and this could add to the explanation that toxoplasmosis could increase the severity of COVID-19 infection because lymphopenia is a well-established bad prognostic marker for COVID-19 and this was in agreement with Moledina *et al*.[44] who reported that lymphopenia is widely described as a poor prognostic marker for survival from COVID-19 infection also this was concordant with other researchers]who reported the same results [45 & 46].

In our study we observed the occurrence of renal impairment and elevated liver enzymes, bilirubin we were more elevated in the positive IgG group than in the negative group but this was statistically insignificant however this could prove that coinfection of toxoplasmosis with COVID-19 increase the severity of multi-organ affection which was reflected on the length of hospital stay, worsening of the outcome, also this was in agreement with Moledina *et al.* [44] who reported that Renal dysfunction, with raised urea (8.1, 95% CI 7–10.9) vs (5.5, 95% CI 4.9–6.1), increased creatinine (104, 95% CI 97–114) vs (82, 95% CI 77–90) and reduced eGFR (54, 96% CI 45–65) vs (77, 95% CI 67–84) were predictive of mortality. inflammatory markers as CRP, ESR, ferritin, and D-dimer in both groups were elevated but more obvious in *Toxoplasma* IgG negative group and also this was statistically insignificant our explanation for this is that renal impairment was more evident in the positive group which may impair the immune system response also hypoalbuminemia was more evident in the positive group which could affect the ESR also decreased CRP could be explained in the positive group by more severe infection is associated with more interferon level which decreases CRP production from the hepatocytes (Table 2).

In our research we found that ventilation was needed in 55.3% of the patients, Regarding the outcome 30 patients needed ICU admission, 16 were discharged and four patients died and this was concordant with Moledina *et al*.[44]who reported that On admission, increased O2 requirement was linked to increased mortality (36, 95% CI 28–85) vs (21, 95% CI 21–28) in those successfully discharged. Furthermore,

desaturation on admission (<94%) was also linked to increased mortality (95%, CI 92–96%) vs (96, CI 96–96%). In the same line, it was declared that chronic toxoplasmosis was the strongest risk factor for a severe course of COVID-19. It was stronger than the effect of being overweight, cardiovascular disease, or diabetes [40].

In our study, we found that IgG level was positively correlated with the RBCs count and other variables showed a statistically insignificant correlation.

A significant statistical difference was observed between negative and positive *Toxoplasma* IgG groups considering both length of the hospital stay and COVID infection outcome. The length of hospital stay in IgG positive group was more than in negative groups which was statistically significant (Table 3). Additionally, the ICU admission rate was higher among the positive group (72.4%) *vs.* (42.9%) in the negative group. Similarly, the death rate was higher among the positive group (10.3%) *vs.* (4.8%) in the negative group.

CT examination revealed statistically significant differences between positive & negative groups regarding chest findings. The positive group showed more lung affection rather than the negative group. The need for mechanical ventilation was significantly higher among the toxoplasmosis positive group (55.3%) vs. only (19.1%) for the negative group. This was in agreement with a study done previously which reported that IgG levels in COVID-19 patients were higher and this was more evident in severe cases. This could be explained by the “immune exhaustion” induced by COVID-19 that enhanced the reactivation of chronic toxoplasmosis [47]. Also this was concordant with other former studies [48, 49, 50]. Those studies reported the same results and their explanation for this was reached by doing a Flowcytometric analysis which was performed using CD3 gating to include both CD4 and CD8+ lymphocytes that are commonly involved in both infections. The recorded increased lymphocytic expression of PD-1 with combined infections was the highest in severe patients.

However, this was not in agreement with a recent study which found a negative correlation between toxoplasmosis and COVID-19 infection even though it appears that the interaction between COVID-19 and toxoplasmosis is mediated by GDP per capita and spatial effects [51]. Also, this was not in agreement with a study done in

2017 which reported that toxoplasmosis could inhibit viral replication both in vivo and vitro [52]. Furthermore, the recent results of Montazeri *et al.* [53] were contrary to ours, as they stated a high rate of latent *T. gondii* infection among COVID-19 with different severity. However, there is no significant relationship between latent *T. gondii* infection and COVID-19 severity and outcomes.

## Conclusion:

We concluded that *T. gondii* prevalence and activity were higher in severe/critical cases and were associated with increased hospital stay and worse outcomes. So *T. gondii* infection can be considered an unrecognized independent risk factor for the severity and bad outcome of COVID-19 so, *Toxoplasma*-infected individuals should have the priority in COVID-19 vaccination programs.

**Power of the study**

Trial of correlating untested widely spread pathogen (toxoplasmosis) and its impact on COVID-19 infection

**Weakness of the study**

The low number of involved patients and inability to test for different cytokines which were tested in different studies.

## References

1. Song F, Shi N, Shan F, Zhang Z, Shen J, Lu H, et al. Emerging 2019 novel coronavirus (2019-nCoV) pneumonia. Radiology 2020, 295; 1:210-217.
2. World Health Organization. Modes of transmission of virus causing COVID-19: implications for IPC precaution recommendations: scientific brief, 27 March 2020.
3. Zaim S, Chong JH, Sankaranarayanan V, Harky A. COVID-19 and multi-organ response. Curr Probl Cardiol 2020; 45(8):100618.
4. Epidemiology Working Group for NCIP Epidemic Response, Chinese Centre for Disease Control and Prevention. The epidemiological characteristics of an

outbreak of 2019 novel coronavirus diseases (COVID-19), China, 2020. China CDC Weekly 2020; 2(8):113-122.

5. World Health Organization. Coronavirus disease (COVID-19), Geneva; 2019 (Situation report, 67).
6. Mehta P, McAuley DF, Brown M, Sanchez E, Tattersall RS, Manson JJ. COVID-19: consider cytokine storm syndromes and immunosuppression. Lancet 2020; 395(10229):1033-1034.
7. Xu Z, Shi L, Wang Y, Zhang J, Huang L, Zhang C, et al. Pathological findings of COVID-19 associated with acute respiratory distress syndrome. Lancet Respir Med 2020; 8(4):420-422.
8. Jain U. Effect of COVID-19 on the organs. Cureus 2020; 12(8):e9540.
9. World Health Organization. Coronavirus disease 2019 (COVID-19). Geneva; 2019 (Situation report, 72).

10. Remuzzi A, Remuzzi G. COVID-19 and Italy: what next? Lancet 2020; 395(10231):1225–1228.

11. Cooper D, Eleftherianos I. Parasitic nematode immunomodulatory strategies: recent advances and perspectives. J Pathog 2016; 5(3):58.

12. Vendelova E, Lutz MB, Hrčková G. Immunity, and immune modulation elicited by the larval cestode Mesocestoides vogae and its products. Parasite Immunol 2015; 37(10):493-504.

13. Dautremepuits C, Betoulle S, Paris-Palacios S, Vernet G. Humoral immune factors modulated by copper and chitosan in healthy or parasitized carp (Cyprinus carpio L.) by Ptychobothrium sp. (Cestoda). Aquat toxicol 2004; 68(4):325-38.

14. Wiedemann M, Voehringer D. Immunomodulation, and immune escape strategies of gastrointestinal helminths and schistosomes. Front Immunol 2020;11: 572865.

15. Xu Y, Chen W, Bian M, Wang X, Sun J, Sun H, et al. Molecular characterization, and immune modulation properties of *Clonorchis sinensis*-derived RNASET2. Parasit Vectors 2013; 6(1):1-8.

16. Elliott DE, Summers RW, Weinstock JV. Helminths as governors of immune-mediated inflammation. Int J Parasitol 2007; 37:457-464.

17. Maizels RM, Balic A, Gomez-Escobar N, Nair M, Taylor M, Allen JE. Helminth parasites: masters of regulation. Immunol Rev 2004; 201:89-116.

18. King CL, Medhat A, Malhotra I. Cytokine control of parasite-specific anergy in human urinary schistosomiasis. IL-10 modulates lymphocyte reactivity. J Immunol 1996; 156(12): 4715–4721.

19. Johnston MJG, Macdonald JA, McKay DM. Parasitic helminths: a pharmacopoeia of anti-inflammatory molecules. Parasitology 2008; 136(2):125-147.

20. Wilson MS, Taylor M, Balic A, Finney CAM, Lamb JR, Maizels RM. Suppression of allergic airway inflammation by helminth-induced regulatory T cells. J Exp Med 2005; 202:1199-1212.

21. Bradbury RS, Piedrafita D, Greenhill A, Mahanty S. Will helminth co-infection modulate COVID-19 severity in endemic regions? Nat Rev Immunol 2020; 20(6):342.

22. Rolot M, Dougall AM, Chetty A, Javaux J, Chen T, Xiao X, et al. Helminth-induced IL-4 expands bystander memory CD8+ T cells for early control of viral infection. Nat Commun 2018; 9(1):4516.

23. Shen SS, Qu XY, Zhang WZ, Li J, Lv ZY. Infection against infection: parasite antagonism against parasites, viruses, and bacteria. Infect Dis Poverty 2019; 8(1):49.

24. Bilenko N, Levy A, Dagan R, Deckelbaum RJ, El-On Y, Fraser D. Does co-infection with Giardia lamblia modulate the clinical characteristics of enteric infections in young children? Eur J Epidemiol 2004; 19:877–8813.

25. Teo TH, Lum FM, Ghaffar K, Chan YH, Amrun SN, Tan JJL, et al. Plasmodium co-infection protects against Chikungunya virus-induced pathologies. Nat Commun 2018; 9:3905.

26. Teo TH, Howland SW, Claser C, Gun SY, Poh CM, Lee WW, et al. Co-infection with Chikungunya virus alters trafficking of pathogenic CD8+ T cells into the brain and prevents Plasmodium-induced neuropathology. EMBO Mol Med 2018; 10:121–138.

27. Cavezzi A, Troiani E, Corrao S. COVID-19: hemoglobin, iron, and hypoxia beyond inflammation. A narrative review. Clin Pract 2020; 10(2):1271.

28. Bulchandani VB, Shivam S, Moudgalya S, Sondhi SL. Digital herd immunity and COVID-19. ArXiv 2004.07237v2. https://arxiv.org/abs/2004.07237. Last update on 26 May 2020.

29. Kwok KO, Lai F, Wei WI, Wong SYS, Tang JWT. Herd immunity: estimating the level

required to halt the COVID-19 epidemics in affected countries. J Infect 2020; 80(6): e32-e33.

30. Briggs N, Weatherhead J, Sastry KJ, Hotez PJ. The hygiene hypothesis and its inconvenient truths about helminth infections. PLoS Negl Trop Dis 2016; 10(9): e0004944.

31. Rook GAW. Introduction: the changing microbial environment, Darwinian medicine, and the hygiene hypothesis. In: The Hygiene Hypothesis and Darwinian Medicine. Birkhäuser Basel 2009; 1-27.

32. Gutman JR, Lucchi NW, Cantey PT, Steinhardt LC, Samuels AM, Kamb ML, et al. Malaria and parasitic neglected tropical diseases: potential syndemics with COVID-19? Am J Trop Med Hyg 2020; 103(2):572-577.

33. Calisher CH, Childs JE, Field HE, Holmes KV, Schountz T. Bats: important reservoir hosts of emerging viruses. Clin Microbiol Rev 2006; 19(3):531-545.

34. Tenter AM, Heckeroth AR, Weiss LM. *Toxoplasma gondii*: from animals to humans. *Int J Parasit.* 2000;**30**:1217–1258.

35. Havlíček J, Gašová Z, Smith AP, Zvára K, Flegr J. Decrease of psychomotor performance in subjects with latent "asymptomatic" toxoplasmosis. Parasitology. 2001;122:515–20.

36. Neyer LE, Grunig G, Fort M, Remington JS, Rennick D, Hunter CA. Role of interleukin-10 in the regulation of T-cell-dependent and T-cell-independent mechanisms of resistance to *Toxoplasma gondii*. Infect Immun 1997;65:1675–82.

37. Kaňková Š, Holáň V, Zajícová A, Kodym P, Flegr J. Modulation of immunity in mice with latent toxoplasmosis - the experimental support for the immunosuppression hypothesis of *Toxoplasma*-induced changes in reproduction of mice and humans. Parasitol Res 2010;107:1421–7.

38. Busoni-Gatel D, Dubremetz JF, Werts C. Molecular cross-talk between *Toxoplasma gondii* and the host immune system. M S-Medecine Sciences 2008;24:191–6.

39. Fenoy IM, Chiurazzi R, Sanchez VR, Argenziano MA, Soto A, Picchio MS, et al. *Toxoplasma gondii* infection induces suppression in a mouse model of allergic airway inflammation. PLoS ONE 2012;7:e43420.

40. Flegr J, Stříž I. Potential immunomodulatory effects of latent toxoplasmosis in humans. BMC Infect Dis 2011;11:274.

41. Bradbury RS, Piedrafita D, Greenhill A, Mahanty S . Will helminth co-infection modulate COVID-19 severity in endemic regions? .Nat Rev Immunol 2020; 20:342–342.

42. Yarovinsky F (2014) Innate immunity to *Toxoplasma gondii* infection. Nat Rev Immunol 14:109–121.

43. Fricker-Hidalgo H, Cimon B, Chemla C, Darde ML, Delhaes L, L'Ollivier C, et al. *Toxoplasma* seroconversion with negative or transient immunoglobulin M in pregnant women: myth or reality? A French multicenter retrospective study.J Clin Microbiol, 51 (2013), pp. 2103-2111.

44. Moledina DG, Simonov M, Yamamoto Y, Alausa J, Arora T, Biswas A ,et al. The association of COVID-19 with acute kidney injury independent of severity of illness: a multicenter cohort study. Am. J Kidney Dis 2021;77(4):490–499.e491. doi:10.1053/j.ajkd.2020.12.007

45. Henry BM, de Oliveira MHS, Benoit S, Plebani M, Lippi G. Hematologic, biochemical and immune biomarker abnormalities associated with severe illness and mortality in coronavirus disease 2019 (COVID-19): a meta-analysis. Clin Chem Lab Med 2020; 58(7): 1021- 1028.

46. Yang AP, Liu JP, Tao WQ, Li HM.The diagnostic and predictive role of NLR, d-NLR and PLR in COVID-19 patients. Int Immunopharmacol 2020;84:106504.

47. Bhadra, R, Gigley, JP, Weiss, LM, Khan, IA. Control of Toxoplasma reactivation by rescue of dysfunctional CD8+ T-cell response via PD-1-PDL-1 blockade. Proc. Natl. Acad Sci USA 2011;108 (22): 9196- 9201.

48. Moretto, MM, Hwang, S, Khan, IA. Downregulated IL-21 response and T follicular helper cell exhaustion correlate with compromised CD8 T cell immunity during chronic toxoplasmosis. Front Immunol 2017;8: 1436.

49. Hwang YS, Shin JH, Yang JP, Jung BK, Lee SH, Shin EH. Characteristics of infection immunity regulated by *Toxoplasma gondii* to maintain chronic infection in the brain. Front Immunol 2018; 5 (9):158

50. Xiao J, Li Y, Yolken RH, Viscidi RP. PD-1 immune checkpoint blockade promotes brain leukocyte infiltration and diminishes cyst burden in a mouse model of *Toxoplasma* infection. J Neuroimmunol 2018;319: 55- 62.

51. Jaroslav Flegr. Toxoplasmosis is a risk factor for acquiring SARS-CoV-2 infection and a severe course of COVID-19 in the Czech and Slovak population: a preregistered exploratory internet cross-sectional study. Parasit Vectors 2021; 14: 508

52. Weeratunga P, Herath TUB, Kim TH, Lee HC , Kim JH , Lee BH. Dense Granule Protein-7 (GRA-7) of *Toxoplasma gondii* inhibits viral replication in vitro and in vivo. J Microbiol 2017; 55, 909–917.

53.Montazeri M, Nakhaei M , Fakhar M, Pazoki H, Pagheh A, Nazar E, *et al.* Exploring the Association Between Latent *Toxoplasma gondii* Infection and COVID-19 in Hospitalized Patients: First Registry-Based Study. Acta Parasit 2022; **67**:1172–1179

**To cite this article**: Asmaa A. Elkholy, Ghada H. Omar , Rasha Omar , Amira Mohamady , Rabab E. Omar , Amira E. Zaki , Eman M. Araby, Seham gouda amen, Ahmed ezzat . Does Latent Toxoplasmosis Impacts SARS-CoV-2 Infection Severity and Outcome: A Cross-Sectional Study in Benha, Egypt. BMFJ 2023;40(1):80-96.