Institute of Parasitology, Biology Centre CAS
Folia Parasitologica 2024, 71: 008
doi: 10.14411/fp.2024.008

Folia Parasitologica
http://folia.paru.cas.cz

Research Article OPEN ACCESS

Impact of latent toxoplasmosis on pneumonic and non-pneumonic COVID-19 patients with estimation of relevant oxidative stress biomarkers

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Abstract: Susceptibility to COVID-19, the most devastating global pandemic, appears to vary widely across different population groups. Exposure to toxoplasmosis has been proposed as a theory to explain the diversity of these populations. The aim of the present study was to investigate the possible association between latent toxoplasmosis and COVID-19 and its probable correlation with markers of oxidative stress, C-reactive protein (CRP) and ferritin. In a case-control study, blood samples were collected from 91 confirmed (48 non-pneumonic; NP, and 43 pneumonic; P) COVID-19 patients and 45 healthy controls. All participants were tested for IgG anti-*Toxoplasma gondii* antibodies and oxidative stress markers (nitric oxide [NO], superoxide dismutase [SOD] and reduced glutathione [GSH]), and CRP and serum ferritin levels were determined. In COVID-19 patients, IgG anti-*T. gondii* antibodies were found in 54% compared to 7% in the control group, with the difference being statistically significant ($P < 0.001$). However, no significant correlation was found between the severity of COVID-19 and latent *T. gondii* infection. Latent toxoplasmosis had a strong influence on the risk of COVID-19. NO and SOD levels were significantly increased in COVID-19 patients, while GSH levels decreased significantly in them compared to control subjects ($P < 0.001$ for both values). CRP and ferritin levels were also significantly elevated in P COVID-19 patients infected with toxoplasmosis. This is the first study to look at the importance of oxidative stress indicators in co-infection between COVID-19 and *T. gondii*. The high prevalence of latent toxoplasmosis in COVID-19 suggests that *T. gondii* infection can be considered a strong indicator of the high risk of COVID-19.

Keywords: *Toxoplasma gondii*, SARS-CoV-2, antioxidants, C-reactive protein, ferritin.

Toxoplasma gondii (Nicolle et Manceaux, 1908) is the most prevalent intracellular parasite on the planet which can infect, survive and multiply in almost all mammalian cells, and causes toxoplasmosis (Hemphill et al. 2019). On a worldwide scale, this parasite infects almost one-third of all people globally (Galván-Ramírez et al. 2023).

Toxoplasmosis has been recognised as one of the life-long parasitic diseases which depend mainly on appropriate immune responses to limit the parasite growth. In immunocompetent individuals, the parasite rapidly resolves into quiescent tissue cysts which continue to exist asymptomatically in host tissues (Halonen and Weiss 2013). However, this parasite is thought to be opportunistic and deadly in those with an immune breakdown (Sina et al. 2021).

The pathophysiology that occurs during *T. gondii* infection is mostly correlated to oxidative stress which results in excessive free radicals' production and increased antioxidant activity is considered one of the host cells' protective mechanisms (Parlog et al. 2015).

COVID-19, the worldwide pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has triggered an exceptionally significant social and economic attention around the world (Nicola et al. 2020). According to the WHO report until July 12, 2023, there were 767,972,961 confirmed cases of COVID-19, with 6,950,655 deaths. To date in Egypt, there have been 516,023 confirmed cases and 24,830 deaths from COVID-19 (World Health Organisation [WHO] 2023).

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In human populations, COVID-19 manifests as febrile respiratory disease ranging from initial symptoms of a common cold to severe pneumonia, that may progress to fatal respiratory failure (Meftahi et al. 2020, Paules et al. 2020). The immunological response to COVID-19 is effectively mediated by specific T cells. In the early stage of infection, the massive lymphocyte activation is followed by progressive lymphopenia, which is known as 'lymphocyte exhaustion'. This leads to a massive increase in the viral load and may end in the loss of a patient's life. Patients may develop a systemic inflammatory syndrome ''cytokine storm syndrome'', a strong uncontrolled immunological response in severe cases of COVID-19 (Sinha et al. 2020, Tay et al. 2020, Zumla et al. 2020, El-Sayed et al. 2021).

The factors prompting heterogeneous populations' susceptibility in this pandemic are still questionable. Evidence suggests that the diversity of clinical manifestations is mainly linked to the diversity of the immune response (Sharaf-El-Deen et al. 2021). Moreover, some researchers hypothesised that some factors could produce non-specific partial protection against the infection such as Bacillus Calmette-Guerin (BCG) vaccination and exposure to tuberculosis, as well as certain infections (Jankowiak et al. 2020, Sala and Miyakawa 2020). It is interesting to note a reversible association between COVID-19 incidence and several parasite illnesses including intestinal helminths, schistosomiasis, malaria, and cutaneous leishmaniasis (Hemphill et al. 2019, Iesa et al. 2020, Bamorovat et al. 2022).

So far, it is doubtful if there is possible relation between COVID-19 and toxoplasmosis. According to certain studies, toxoplasmosis has negative correlation to incidence of COVID-19. Indeed, this intracellular parasite exhibits broad antiviral effects by secreting into host cells a 'Dense Granule Protein-7' which has immune stimulatory effects and inhibits replication of virus (Fekadu et al. 2010, Flegr 2013, Weeratunga et al. 2017). Additionally, apicoplast proteins exhibit remarkably immune-mediated properties (Can et al. 2020).

However, some other authors claim that toxoplasmosis worsens COVID-19 symptoms. Theoretically, both pathogens are intracellular and share the immunological reaction known as 'lymphocytic exhaustion', which could explain this positive correlation. Higher production of certain stimulated cytokines in *T. gondii* infection may also exacerbate COVID-19 severity (Bradbury et al. 2020, Jankowiak et al. 2020, Roe 2021).

In this regard, the main objective of the current research is to estimate the seroprevalence of *T. gondii* infection amongst patients with pneumonic and non-pneumonic COVID-19, and to explore whether latent *T. gondii* co-infection affects positively or negatively the risk of COVID-19, as well as C-reactive protein, serum ferritin and oxidative stress markers.

MATERIALS AND METHODS

Study population, data and sample collection

This hospital-based case control study involved a total of 136 individuals, categorised into two distinct groups. Patients in the cases group (91/136) included those who had COVID-19 infection during the period from November 2021 to March 2022. These patients were admitted to the isolation specialised centre in Beni-Suef University Hospital designated for treating COVID-19 patients. The control group consisted of 45 healthy volunteers who were age- and sex- matched with the selected patients. The individuals in the control group did not have any immunodeficiency illnesses and tested negative for COVID-19 infection.

Table 1. Demographic and clinical data of healthy controls, non-pneumonic, and pneumonic COVID-19 patients with seroprevalence of latent *Toxoplasma gondii* infection in all groups.

	Healthy controls (n = 45)	Non pneumonic COVID-19 (n = 48)	Pneumonic COVID-19 (n = 43)	P
Gender	n (%)	n (%)	n (%)	
Female	26 (58)	30 (63)	21(49)	$P > 0.05$
Male	19 (42)	18 (38)	22 (51)	
Age: 20–40y	20 (44)	26 (54)	20 (47)	
41–60y	19 (42)	16 (33)	12 (28)	
61–80y	6 (13)	6 (13)	11 (26)	$P > 0.05$
Mean & SEM	44 ± 2[a]	41 ± 2[a]	46 ± 2[a]	
DM				
Yes	-	5 (10)	8 (19)	$P > 0.05$
No		43 (90)	35 (81)	
HTN				
Yes	-	5 (10)	9 (21)	$P > 0.05$
No		43 (90)	34 (79)	
Fever				
Yes	-	29 (60)	26 (61)	$P > 0.05$
No		19 (40)	17 (40)	
Cough				
Yes	-	26 (54)	27 (63)	$P > 0.05$
No		22 (46)	16 (37)	
Dyspnea				
Yes	-	10 (21)	21 (49)	$P<0.001$
No		38 (79)	22 (51)	
Headache				
Yes	-	23 (48)	3 (7)	$P > 0.05$
No		25 (52)	40 (93)	
Body pain				
Yes	-	25 (52)	18 (42)	$P<0.001$
No		23 (48)	25 (58)	
GIT symptoms				
Yes	-	29 (60)	17 (40)	$P > 0.05$
No		19 (40)	26 (61)	
Anti- Toxoplasma IgG$^+$	3 (7)[a]	24 (50)[b]	25 (58)[b]	$P<0.001$

SEM: Standard error means. Means which share the same superscript symbol (s) are not significantly different (P >0.05)

Patients who had received a definite diagnosis of SARS-CoV-2 infection, based on the published interim guidelines of the World Health Organisation (WHO 2020), were incorporated in the study. Positive nasopharyngeal swab tests were confirmed through the utilisation of real-time polymerase chain reaction (RT-PCR) for all the participants considered. The most common strain of COVID-19 in this period of time was Omicron.

According to the classification provided by WHO, the severity of COVID-19 infection was categorised as mild, moderate and severe. The mild category was defined as having typical symptoms without signs of viral pneumonia or low oxygen levels. Cases were regarded moderate or severe if there was clinical and radiological evidence of pneumonia (WHO 2020).

We categorised our patients into two main groups (non-pneumonic; NP vs pneumonic patients; P). Compared to normal chest X-rays, chest CT was more sensitive and allows for the identification of consequences beyond pulmonary involvement and the suggestion of other diagnosis. Airspace opacities (consolidations

Table 2. Distribution and associated risk factor of *Toxoplasma gondii* infection in P COVID-19 patients.

Variable	n.	P COVID-19	%	Control	%	X^2	OR	RR	r	P
Anti-Toxo IgG+	28	25	89.3	3	10.7	26.85	19.44	2.97	0.55	0.001
Anti-Toxo IgG-	60	18	30.0	42	70.0					

X^2 – chi-square; OR – odds ratio; P – probability value; RR – relative risk; r – correlation

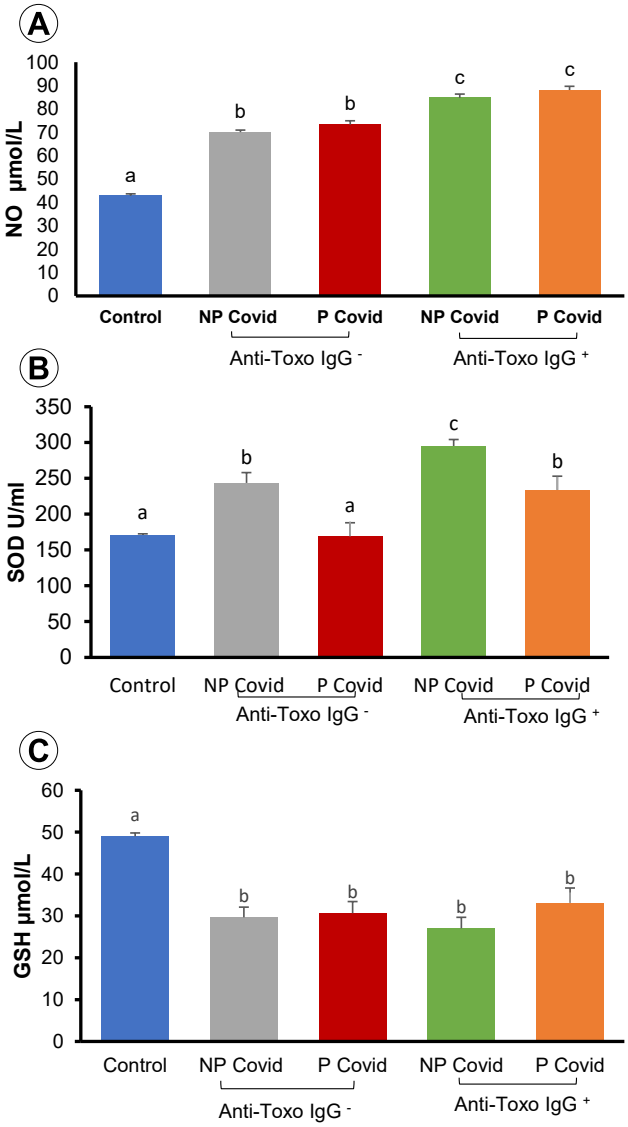

Fig. 1: NO (A), SOD (B) and GSH (C) levels of healthy controls, anti-*Toxoplasma gondii* IgG^- (NP^- and P^-) and anti-*T. gondii* IgG^+ (NP^+ and P^+) COVID-19 patients. Means which share the same superscript symbol (s) are not significantly different (P > 0.05). NO – nitric oxide; SOD – superoxide dismutase; GSH – reduced glutathione; NP – non-pneumonic; P – pneumonic.

and/or ground-glass opacities), which were usually bilateral, peripheral, and mostly found in the lower fields, were the most frequent radiologic findings in COVID-19 patients (Martínez Chamorro et al. 2021).

By designing a questionnaire checklist, sociodemographic data (gender, age, previous medical records and use of drugs), clinical data (COVID-19 symptoms and their duration, respiratory rate, O_2 saturation, presence or absence of pneumonia, grade of severity, and treatment protocol), and prognostic factors (D dimer, C-reactive protein, serum ferritin, complete blood count) were filled out for each patient. All other related information was provided from the patient's physicians and medical examination records.

Whole blood (5 ml) sample was drawn from each participant in the study using venipuncture under sterile conditions. The blood sample was collected in Wasserman tube, centrifuged for 10 min at 4,000 rpm to separate the serum and, then kept in Eppendorf tubes at -20°C. The serum sample was used to detect IgG anti-*T. gondii* antibodies, as well as to assess the oxidative stress markers.

The present study was approved by the Research Ethics Committee of Faculty of Medicine, Beni-Suef University (FM-BSU REC), with approval No. FMBSUREC/12022023 in accordance with principles of the Helsinki Declaration. An informed consent was obtained from all participants after explaining the purpose of the study, and contents of the questionnaire.

Serological assessment of *T. gondii* infection

Following the manufacturer's recommendations, serum samples from each participant were screened for specific IgG anti-*T. gondii* antibodies using a commercially available ELISA (Pre Check Bio, Inc. Kyunggi-Do, Korea). Each plate's optical density (OD) was measured at a wavelength of 450 nm, and the cut-off value was calculated from the equation mentioned by the manufacturer. The test has 99.9% sensitivity and 100% specificity rate.

Assessment of oxidative stress markers

Nitric Oxide (NO) level was calorimetrically measured by an indirect technique based on the measurement of nitrite concentration as an indicator of NO production through the addition of Griess' reagents (Jabłonska et al. 2007). By using a standard curve and spectrophotometric analysis at 540 nm, nitrite concentrations were calculated. NO were expressed as µmol/l following the manufacturer's instructions (Elabscience Co., Houston, Texas, USA).

Superoxide dismutase (SOD) activity was measured with (Cu-Zn-SOD/ Mn-SOD) activity assay kits (hydroxylamine method) following the manufacturer's instructions (Elabscience Co.). Absorbance was read spectrophotometrically at 550 nm (Cristiana et al. 2014).

Reduced glutathione (GSH) level was spectrophotometrically estimated following the procedure described by Ellman (1959). This procedure depends on the observation that sulfhydryl compounds (GSH) react with Ellman's reagent, 5'5'-dithiobis (2-nitrobenzoic acid), to form a rather persistent yellow molecule. Absorbance of the sample was determined at 412 nm and expressed as µmol/l, as per the guidelines provided by the manufacturer (Elabscience Co.).

Statistical analysis

Analysis of the statistics was done using SPSS version 22.0 software (Chicago, Illinois, USA). The association between

Table 3. Distribution and associated risk factor of *Toxoplasma gondii* infection in NP COVID-19 patients.

Variable	n.	NP COVID-19	%	Control	%	X^2	OR	RR	r	P
Anti-Toxo IgG+	27	24	88.9	3	11.1	21.17	14	2.4	0.477	0.001
Anti-Toxo IgG-	66	24	36.4	42	63.6					

X^2: chi-square, OR: odds ratio, RR: relative risk, r: correlation

categorical variables was evaluated by Chi-squared test (X^2). To detect the significant statistical differences between groups ($P < 05$), one way analysis of variance and the Duncan's multiple range test were processed. The findings were presented as mean ± SEM. To determine the associations between the variables, simple linear correlation analysis was conducted using the Pearson technique (IBM SPSS).

RESULTS

In the present study, 48 (53%) out of 91 patients with COVID-19 had mild (non-pneumonic), and 43 (47%) had moderate (pneumonic) COVID-19. Males represented 40 (44%) of cases, while 51 (56%) were females with non-significant statistical difference between healthy control, P COVID-19 and NP COVID-19 patients ($P > 0.05$). Age of all participants ranged from 20 to 80 years. There was no statistical difference in mean and SEM of age values between healthy individuals, P COVID-19 and NP COVID-19 patients ($P > 0.05$). Age values of all participants age are illustrated in Table 1.

There was no significant association between the past history of *diabetes mellitus* or hypertension and COVID-19 severity ($P > 0.05$). Dyspnea and body pain were the only clinical symptoms that had a marked association with COVID-19 severity ($P < 0.001$). Our findings showed that seroprevalence of anti-*Toxoplasma gondii* IgG was 54% (49/91) in COVID-19 patients compared to 7% (3/45) in healthy controls with a high statistical difference ($P < 0.001$). Although anti-*Toxoplasma* IgG seropositivity was higher in P COVID-19 (58%) patients than in NP COVID-19 patients (50%), the difference between both groups was statistically non-significant (Table 1).

Tables 2 and 3 show the prevalence of latent toxoplasmosis in P and NP COVID-19 patients compared to healthy control group. Anti-*T. gondii* IgG were detected in 25/28 (89%) in P COVID-19 patients and in 24/27 (89%) in NP COVID-19 ones. This single variable analysis suggested that latent toxoplasmosis was more prevalent in COVID-19 cases and considered a high statistically significant predictor for P and NP COVID-19 patients ($P = 0.001$, 0.001/RR = 2.97, 2.4/OR = 19.44, 14, respectively).

As shown in Fig. 1, NO levels raised significantly in patients with COVID-19 in both groups (anti-*T. gondii* IgG− and IgG+) compared to healthy individuals ($P < 0.001$). Both NP+ and P+ levels were significantly higher than NP- and P- ($P < 0.001$). SOD levels also increased significantly in patients with COVID-19 in NP-, NP+, and P+ compared to control group. Moreover, SOD levels markedly increased in both NP+ and P+ ($P < 0.01$ and $P < 0.001$, respectively) compared to those in P- and healthy controls. In contrast, GSH decreased significantly in patients with COVID-19 in both groups (anti-*T. gondii* IgG− and IgG+) compared to healthy subjects ($P < 0.001$).

As illustrated in Fig. 2, C-reactive protein (CRP) and ferritin levels were markedly elevated in COVID-19 NP+, P+, and P- patients compared to control group ($P < 0.001$). Meanwhile, CRP levels showed no statistical difference between P +, NP+ and P− ($P > 0.05$). Also, ferritin levels were not statistically different between NP−, P− and NP+, but they were statistically different between P− and P+ groups ($P < 0.001$).

Fig. 3 shows the levels of anti-*T. gondii* IgG correlations in COVID-19 patients. It was positively correlated with NO (r = 0.42, $P < 0.001$), SOD (r = 0.408, $P < 0.001$), and GSH levels (r = 0.168, $P > 0.05$), but negatively correlated with CRP (r= -0.176, $P > 0.05$), and ferritin levels (r = -0.11, $P > 0.05$).

DISCUSSION

On a global level, little is known regarding the relation between *Toxoplasma gondii* infection and COVID-19 outcomes. Patients with COVID-19 who have chronic illnesses or using immunosuppressive medications are at a higher risk of catching opportunistic infections. Therefore, early diagnosis, prevention and treatment are crucial (Abdoli et al. 2022). Wolday et al. (2021) proposed that co-parasitism indicates severity of COVID-19 cases. Another study published by Bradbury et al. (2020) suggested that helminthic co-infections have a prospective role in hyperinflammatory modulation in COVID-19.

The present study revealed non-statistically significant difference in mean age and SEM of age values between healthy individuals, NP COVID-19 and P COVID-19 patients, the same as reported by Montazeri et al. (2022) who detected non-significant relation between mean age and COVID-19 severity. The participants' gender did not significantly correlate with COVID-19 severity, similarly as demonstrated by Montazeri et al. (2022). This study did not detect a significant association between the past history of *diabetes mellitus* and hypertension and COVID-19 severity and that goes in hand with the results of Montazeri et al. (2022).

In the present study, dyspnea and body pain were the only clinical symptoms that had a marked association with COVID-19 severity. This contrasts with the results of Ghaffari et al. (2021) and Geraili et al. (2023) who did not find any significant correlation between *T. gondii* IgG seropositive results and clinical manifestations amongst COVID-19 patients including dyspnea, muscle and body pains.

The obtained results revealed that 54% of COVID-19 patients were positive for anti-*T. gondii* IgG, with less prevalence rate in mild NP COVID-19 (50%, OR= 14) than moderate P COVID-19 cases (58%, OR= 19.44) compared to healthy controls (7%). This relationship was of high statistical difference between cases and control and with no statistical correlation between NP and P COVID-19 pa-

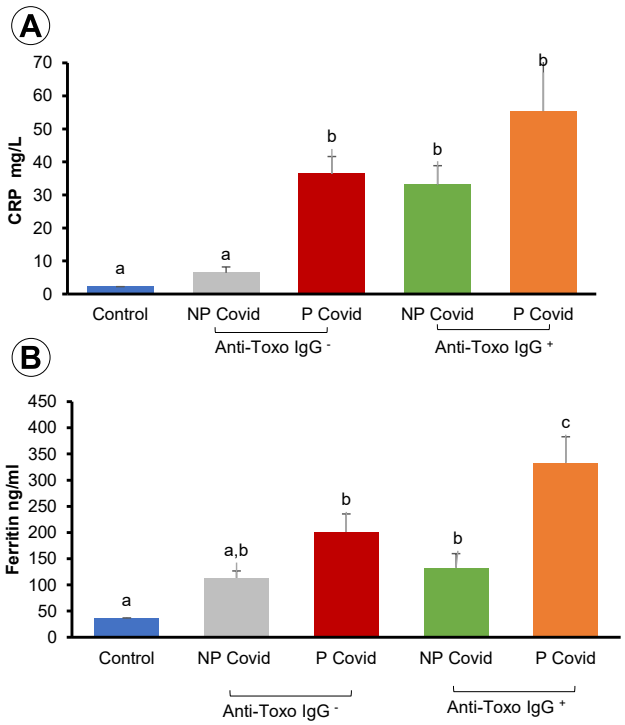

Fig. 2. CRP (A), and Ferritin (B) levels of healthy controls, anti-*Toxoplasma gondii* IgG^{-} (NP^{-} and P^{-}) COVID-19 patients and anti-*T. gondii* IgG^{+} (NP^{+} and P^{+}) COVID-19 patients. Means which share the same superscript symbol(s) are not significantly different ($P > 0.05$). CRP – C-reactive protein.

tients. Abed and Kalaf (2023) reported latent toxoplasmosis in 53% in COVID-19 patients, i.e. very similar to our results. In contrast, Ghaffari et al. (2021) and Montazeri et al. (2022) detected higher prevalence rates of latent *T. gondii* infection (84% and 82%, respectively) amongst COVID-19 Iranian patients. Ali and Saheb (2022) and Geraili et al. (2023) reported lower detection rates (33% and 26%) of anti-*T. gondii* IgG antibodies in COVID-19 patients in Iraq and Iran. Our findings and other studies indicate a negative correlation between latent toxoplasmosis and COVID-19 severity (Jankowiak et al. 2020, Flegr 2021, Ghaffari et al. 2021, Montazeri et al. 2022, Geraili et al. 2023).

On the other side, our results were in contrast to a recent Egyptian study that considered chronic toxoplasmosis as an independent risk factor for COVID-19 severity (Sharaf-El-Deen et al. 2021). Existing data are controversial in different areas of the world. Variations in the level of *T. gondii* endemicity in different geographical regions as well as the predominant *T. gondii* genotypes and variants of COVID-19 in different pandemic periods seem to be the main causes for these contradictory findings.

Many research studies have discussed the correlation between COVID-19 and oxidative stress pathogenesis (Yaghoubi et al. 2022, Golabi et al. 2022, Binici et al. 2023). One of the theories that explain the pathogenesis induced by *T. gondii* and its intracellular persistence includes oxidative stress production and reactive oxygen radicals (Denkers et al. 2003). In fact, the parasite overcomes the antioxidant enzymatic defence mechanisms which protect the host cells against the rise of free radicals during toxoplasmosis (Parlog et al. 2015). However, there are scarce data about the oxidative capacity and antioxidative profile in humans infected with *T. gondii* especially amongst COVID-19 patients.

Infection with *T. gondii* is linked to increased NO level, which is generated to control the infection. NO leads to death of tachyzoites and/or stimulation of heat-shock protein 70 production in both non-virulent and virulent tachyzoites strains, helping in their conversion to bradyzoites and formation of tissue cysts (Miller et al. 1999, Tonin et al. 2015). Our findings revealed significant elevation of serum NO levels in patients with P and NP COVID-19 in both groups (anti-*T. gondii* IgG− and IgG+) as compared with control group.

However, P and NP COVID-19 anti-*T. gondii* IgG+ revealed more elevation as compared to anti-*T. gondii* IgG-. In parallel with our obtained results, Marchioro et al. (2018) detected significantly high NO levels in pregnant women infected with toxoplasmosis, and Kiran et al. (2019) observed significantly higher NO levels in *T. gondii*-infected patients as well. On the other side, no significant differences were observed between serum levels of NO in COVID-19-infected patients compared with the healthy subjects (Yaghoubi et al. 2022). These data have been supported by a marked positive correlation between anti-*T. gondii* IgG and NO levels.

Another antioxidant enzyme is SOD, which prevents formation of the hydroxyl radicals by the detoxification of hydrogen peroxide (Halliwell 2012), and prohibits the onset of free radical chain reactions, which is necessary for intracellular survival of *T. gondii* (Mohammed et al. 2020). In the present study, SOD activity increased significantly in NP-, NP+ and P+ COVID-19 patients as compared with healthy individuals. This finding is in contrast to the results of Mohammed et al. (2020) who found a significant decrease in SOD in sera of *T. gondii*-infected women, compared with the control group. Al-Khshab (2010) pointed out that no changes occurred in the serum SOD activity in the patients infected with *T. gondii* neither when associated with the increase in oxidative stress nor in the case of severe parasitemia. Mehri et al. (2021), Golabi et al. (2022) and Binici et al. (2023) also reported higher serum concentrations of SOD in COVID-19 patients than in healthy persons. In an experimental study, Türkoğlu et al. (2018) found elevation in SOD level in *T. gondii*-infected rats. In contrast, recent studies on COVID-19-infected patients detected a decrease in antioxidant activity and SOD concentration amongst patients with severe form of the disease (Abbasi et al. 2018, Yaghoubi et al. 2022). SOD is an antioxidant protein dependent on zinc. Zinc depletion in COVID-19 infection leads to dysfunction of zinc-dependent antioxidant proteins such as SOD. Moreover, as SOD is considered as one of the most important mechanisms of reactive oxygen species detoxification, its high serum levels revealed in the present study may neutralise the oxidative stress present in SARS-CoV-2 infection through this regenerative mechanism (Golabi et al. 2022).

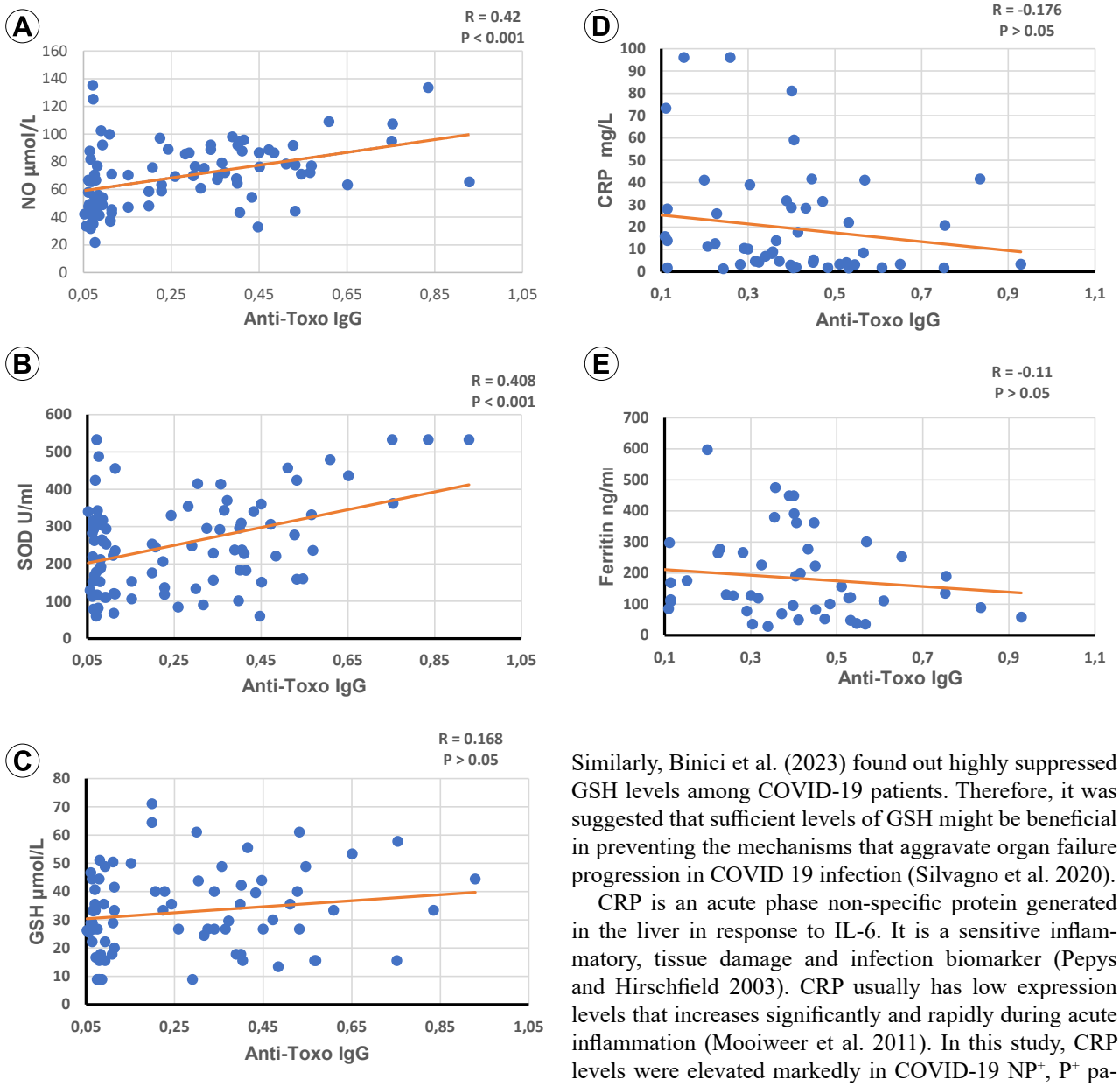

Fig. 3. Graphical correlations between the levels anti-*Toxoplasma gondii* (Anti-Toxo IgG) and NO (A), SOD (B), GSH (C), CRP (D) and ferritin (E). Values were considered significantly different at $P < 0.05$, $P < 0.01$ and $P < 0.001$.

GSH has variable functions in the protection of tissues against exogenous and endogenous oxidative damage by detoxification of lipid peroxidation products and binding to oxygen reactive species (Halliwell 2012, Pisoschi and Pop 2015). A decrease in GSH levels was detected in *T. gondii* infected patients, which proposed the oxidant-antioxidant imbalance as one of tissue damage mechanisms (Khaleel et al. 2020). This study revealed significant decline in GSH serum levels in patients with COVID-19 with anti-*T. gondii* IgG− and IgG+ in comparison to healthy group. Similarly, decreased levels of GSH were noticed in patients with ocular toxoplasmosis and pregnant females infected with *T. gondii* in comparison with seronegative subjects (Mohammed et al. 2020, Paraboni et al. 2022). Similarly, Binici et al. (2023) found out highly suppressed GSH levels among COVID-19 patients. Therefore, it was suggested that sufficient levels of GSH might be beneficial in preventing the mechanisms that aggravate organ failure progression in COVID 19 infection (Silvagno et al. 2020).

CRP is an acute phase non-specific protein generated in the liver in response to IL-6. It is a sensitive inflammatory, tissue damage and infection biomarker (Pepys and Hirschfield 2003). CRP usually has low expression levels that increases significantly and rapidly during acute inflammation (Mooiweer et al. 2011). In this study, CRP levels were elevated markedly in COVID-19 NP^+, P^+ patients and P^- patients as compared to those in healthy group and showed non-statistically different relation between P+, NP^+, and P^- patients. Our results are in agreement with those of Ali and Saheb (2022) who found that the CRP mean value in COVID-19 cases having toxoplasmosis was greater than its mean level in controls with a statistically significant relation.

Previous studies indicated a significant increase in CRP levels between 20 to 50 mg/l in COVID-19 patients (Chen et al. 2020, Mo et al. 2021). CRP in concentration of 18.8 mg/l was observed in patients suffering from mild symptoms, while patients having more severe manifestations showed CRP of 39.4 mg/l and these values are similar to our findings (Gao et al. 2020). Mehri et al. (2021) and Golabi et al. (2022) found that CRP was higher amongst COVID-19 patients than in controls. On the other hand, CRP showed a significant relation with COVID-19 outcomes as discussed by Montazeri et al. (2022). Levels of anti-*T. gondii* IgG in COVID-19 patients were negatively correlated with CRP. This finding is expected as when

the tissue damage or acute inflammation subsides, CRP concentration starts to fall, making it a vital biomarker for monitoring the severity of the disease (Young et al. 1991).

Ferritin is well known as iron-storing protein; the normal iron level reverses the serum ferritin level, so it helps in the diagnosis of iron deficiency anemia. During viral infections, ferritin level increases being a biomarker of viral replication (Baraboutis et al. 2020, Li et al. 2020). Severe COVID-19 patients reported rising concentrations of ferritin as a result of a cytokine storm and subsequent hemophagocytic lymphohistiocytosis (Velavan and Meyer 2020). In the prvious studies, ferritin levels elevated markedly in COVID-19 NP^+, P^+ and P^- individuals compared to those in healthy subjects. Ferritin levels were not statistically different between NP^-, P^-, and NP^+, but it was statistically different between P^- and P^+. This finding is similar to that of Ali and Saheb (2022) who revealed that the mean level of ferritin in COVID-19 patients infected with *T. gondii* increased when compared to control.

Additionally, Binici et al. (2023) revealed higher ferritin and CRP levels in COVID-19 patients before treatment than after treatment. In case of inflammation, the release of the reticuloendothelial system for iron is partially blocked and this leads to an increased rate of ferritin synthesis (Worwood 1990). Levels of anti-*T. gondii* IgG in COVID-19 cases were negatively correlated with ferritin levels. This may be attributed to the recovery of acute inflammation and declining IgG titre (Ali and Saheb 2022). The small sample size of COVID-19 patients in this study was one of its drawbacks. Larger sample sizes from various groups are required for further investigations in order to make more precise health recommendations.

To conclude, this is the first research, to the best of our knowledge, representing the seroprevalence of latent toxoplasmosis in COVID-19 patients in Upper Egypt. The current study concluded that *T. gondii* infection is considered a strong indicator to the high risk of COVID-19 infection; meanwhile latent infection did not significantly correlate with COVID-19 severity. The pneumonic COVID-19 patients infected with toxoplasmosis had considerably higher serum concentrations of CRP and ferritin. Level of anti-*T. gondii* IgG was positively correlated with NO, SOD and GSH levels but negatively correlated with CRP and ferritin levels. Significant increase in NO concentration, SOD activity and marked decline in GSH levels were recorded in patients with *T. gondii*-COVID-19 co-infection as compared to healthy subjects. These findings may explain the pathogenesis and interaction between both infections and may be helpful in diagnosis, prognosis and therapeutic plans. The performance of molecular characterisation for these patients is highly recommended for a better exploration and determination of *T. gondii* genotypes.

Authors' contribution. DAH, RAE, HA and WMA were involved in designing the study. RAE and AMA collected the clinical data. DAH, HA, MAE and WMA conducted the laboratory work. DAH, HA and WMA performed the data analysis. DAH, RAE, HA, MAE, AMA and WMA contributed to writing the paper. All authors have revised the manuscript critically as to its scientific content, edited and approved the final manuscript.

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Received 17 August 2023 Accepted 16 February 2024 Published online 4 April 2024

Cite this article as: Hamdy D.A., Eid R.A., Abdel-Tawab H., El-Badry M.A., Abdallah A.M., El Wahab W.M.A. 2024: Impact of latent toxoplasmosis on pneumonic and non-pneumonic COVID-19 patients with estimation of relevant oxidative stress biomarkers. Folia Parasitol. 71: 008.