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TOXOPLASMOSIS IN VACCINATED COVID-19 PATIENTS

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Abstract

Toxoplasma gondii is associated with viral infections like cytomegalovirus (CMV), and human immunodeficiency virus (HIV), and an important a factor in SARS-COV-2 global pandemic. This study investigated the potential attribution of *T. gondii* as a risk factor in COVID-19 infection after vaccination and to detect associated hematological and biochemical parameters.

Out of 375 COVID-19 patients enrolled in the study, latent toxoplasmosis was detected in 108/375 (28.8%) patients. Regarding hematological parameters, a significant difference was reported in white blood cells, lymphocyte, and neutrophil counts. Biochemical parameters, LDH (426.5±446.4), ferritin (453.71±283.36), and D-dimer (603.43±519.13) were significant among cases. The current work highlights the role of toxoplasmosis in COVID infections following vaccination through the changes in the hematological and biochemical parameters.

Keywords: *Toxoplasma gondii*, vaccinated COVID-19, Laboratory diagnosis, Biomarkers.

Introduction

Vaccination is the cornerstone of public health measures against the global COVID-19 pandemic, which gave protection rates of 90% or higher against serious illness and fatality (Fernandes *et al,* 2022). However, the emergence of the Omicron strain and its remarkable transmission speed has presented a new hurdle just a year after vaccinations. Omicron has a higher immune escape rate, is more transmissible, and is less harmful overall. Since vaccines do not provide complete protection against SARS-CoV-2 acquisition, infections may occur in completely immunized persons, who may then transfer the virus to others (Amanatidou *et al,* 2022).

The immune responses elicited by vaccination exhibit heterogeneity among individuals, meaning the same vaccine can confer protection in certain situations but may prove ineffective in others. One potential determinant that may impact the reaction to vaccination is concurrent or previous infection with irrelevant parasites (Wait *et al,* 2020).

Toxoplasmosis is a prevalent parasitic infectious disease that poses a significant global health concern, affecting populations in developing and developed nations (Lindsay and Dubey, 2020), associated with developing various hazardous pathologies, affecting the chest, liver, eye, and serious neurological disorders (Antczak *et al,* 2016). Immunocompromised individuals are susceptible to experiencing lethal complications from toxoplasmosis (Mohammed *et al,* 2021). Anti-activity of *T. gondii* produces toxic substances reactive oxygen intermediates (ROI), and during intracellular infection generating oxidants due to metabolic processes (Mosa *et al,* 2020).

The co-occurrence of COVID-19 and toxoplasmosis has received limited attention with inconsistent findings (Jankowiak *et al,* 2020). Flegr (2021) in the Czech Republic reported that latent toxoplasmosis may be a risk factor for COVID-19. But, Jandaghi *et al.* (2021) in Iran didn't detect significant correlation between COVID-19 and latent toxoplasmosis. Hébert *et al.* (2022) reported that patients have developed toxoplasmosis after being infected or vac-cinated with COVID-19, with increased their susceptibility to toxoplasmic retinochoroiditis. Roe (2022) reported a rise in mortality among COVID-19 patients with both toxoplasmosis

and schizophrenia

The present study aimed to evaluate *Toxoplasma gondii* impact on hematological, and biochemical parameters among vaccinated COVID-19 patients.

Subjects and Methods

Study; this is a case-control study, CDC reported that toxoplasmosis seropositivity among COVID-19 patients was 60% at power of 90%, marginal error of 0.05, and minimal of 375 COVID-positive cases (Bigna *et al,* 2020).

The participants were selected from June 2022 to June 2023. The study was approved by the Institutional Review Board (IRB) of the National Liver Institute, Menoufia University, Egypt (NLI IRB protocol N. 00512/ 2023). Informed written consent was obtained from each one.

n = Zα-12* (PQ) e2 n = sample size, z = standard error with confidence chosen level (1.96), p = proportion detected in reference study (58%), q = 1-p, & e = acceptable sample error (0.05).

Inclusion criteria were positive patients for SARS-COV-2 by RT-PCR, and with fever, cou-gh, sore throat, muscle ache, headache, dyspnea, diarrhea, loss of taste, and loss of smell. The SARS-COV-2 negative ones or with other concurrent acute illnesses were excluded. Demographic data were collected by questionnaire including age, sex, and comorbidities as hyper-tension, diabetes mellitus, heart disease, and bronchial asthma.

Laboratory examination: Blood was withdrawn to separate sera for CBC, C-reactive protein, lactate dehydrogenase (LDH), and ferritin using a particle-enhanced immunoturbidimetric assay on Cobas c501 Roche-Germany. D-dimer levels were assayed by Cobas 6000analyzer, specifically c501 module (Lindahl *et al,* 1999). Also, sera were tested for anti-*T. gondii* IgG & IgM antibodies by ELC kits (COBAS, Roche Diagnostics) with Elecsys TOXIGG (04618815119) and TOXIGM (04618858119) and patients were categorized to a negative toxoplasmosis or negative control and positive toxoplasmosis positive cases.

Statistical analysis: Data were gathered and analyzed using student *t*-test and Mann-Whitney U test for quantitative data and chi-squared or Fisher's Exact test, and Z test for qualitative data, and analyzed using SPSS (Statistical Package for social science) version 23.0 on an IBM-compatible computer (SPSS Inc., Chicago, IL, USA). P <0.05 was considered statistically significant result.

Results

Of 375 COVID-19 patients, 108 (28.8%) served as cases (*T. gondii* seropositive) with ages ranged from 14-80(46.36±15.61) years. Of 267 (71.2%) *T. gondii* seronegative served as negative control with ages ranged from 19-77(45.21±14.22) years without significant differences as to ages or sex.

T. gondii seropositive among vaccinated positive COVID patients were IgM antibodies negative, and 108 (28.8%) were anti-*T. gondii* IgG antibodies positive

Most patients recorded negative for comorbidities; but some cases suffered from hypertension (HTN), diabetes mellitus (DM), asthma, and cardiac problems without significant differences, but 9(8.3%) patients in cases group and 19 (7.1%) in negative control group required hospitalization.

Symptoms were fever followed by others as cough, sore throat, headache, diarrhea, smell loss, taste loss, and pain. Only cough was significant (P <0.001).

Hematological analysis didn't show difference between cases and negative control as to Hemoglobin concentration, which carries oxygen RC indices, and platelet count, but WBCs showed a significant difference (P= 0.005). There was a significant differences in lymphocytes & neutrophils (P<0.001). *T. gondii* caused the lymphocytes reduction (23.21±13.97%) as compared to the control (29.41±12.01%), but elevated neutrophil count (69.88±15.01%) as compared to control (62.55±12.04%). LDH, D-dimer and ferritin levels didn't show significant difference, but CRP was significant elevated in co-infected cases (P < 0.001). Positive or negative anti-

Toxo-IgG with COVID were significant. The death from cases and control was 0.9%. Details were given in tables (1, 2, 3 & 4) and figure (1).

Table 1: Sociodemographic and clinical criteria among cases

Variants	Cases (N = 108)	Control (N = 267)	Test	P value
Age (years) range	14- 80(46.36±15.61)	19-77(45.21±14.22)	0.69	0.49
Male	50 (46.3%)	104 (39.0%)	X^2	
Female	58 (53.7%)	163 (61.0%)	1.71	0.19
No comorbidity	97 (89.8%)	202 (75.7%)	Z= 2.95	0.003
HTN	6 (5.6%)	34 (12.7%)	1.85	0.06
DM	2 (1.9%)	18 (6.7%)	1.65	0.10
Asthma	1 (0.9%)	7 (2.6%)	0.63	0.53
Cardiac	2 (1.9%)	6 (2.2%)	0.15	0.88
Fever	108 (100%)	262 (98.1%)	2.05	0.15
Cough	17 (15.7%)	100 (37.5%)	16.9	<0.001*
Sore throat	49 (45.4%)	143 (53.6%)	2.06	0.15
Headache	72 (66.7%)	200 (74.9%)	2.62	0.11
Diarrhea	5 (4.6%)	33 (12.4%)	5.04	0.03
Loss of smell	11 (10.2%)	50 (18.7%)	4.12	0.04
Loss of taste	8 (7.4%)	46 (17.2%)	6.11	0.01
Muscle ache	16 (14.8%)	86 (32.2%)	1.34	0.51

* Significant differences

Table 2: Hematological parameters among cases

Variants	Cases (N = 108)	Control (N = 267)	T-test	P value
Hb range	8.6-15.8 (12.59±1.58)	8.1-15.6 (12.41±1.62)	1.05	0.30
RBCs range	2.6-6.9 (4.86±0.71)	2.6-6.9 (4.83±0.63)	0.42	0.68
HCT range	27-49.7 (39.13±4.87)	27-48 (38.71±4.45)	0.79	0.43
MCV range	63.9- 99 (80.44±6.14)	64-99 (80.13±6.07)	0.45	0.65
MCH range	20-32 (26.61±2.69)	20-90(26.71±4.87)	0.20	0.84
MCHC range	29-38.1(32.59±1.55)	29- 35 (32.34±1.49)	1.46	0.14
WBCs range	2.7-28.1 (9.06±4.70)	2.7-17.2 (7.41±2.78)	U=2.78	0.005
Platelets range	22-569 (243.98±95.93)	32-426 (234.41±74.75)	U=0.81	0.41
Lymphocyte range%	4-65 (23.21±13.97)	5- 65 (29.41±12.01)	U=4.64	<0.001*
Lymphocyte range no.	399-4500 (1725.6±806.7)	246-4900(2063.51±892.9)	U=3.48	<0.001*
Neutrophil range %	27-94 (69.88±15.01)	25-86 (62.55±12.04)	4.52	<0.001*
Neutrophil range no.	400-24447(6743.5±4554.0)	400-15000 (4760.2±2334.9)	U=3.77	<0.001*

* Significant differences

Table 3: Biochemical and physiological parameters among cases

Variants	Cases (N = 108)	Control (N = 267)	Test	P value
CRP range	0-200 (34.52±44.85)	0.1-242 (24.33±36.78)	U=1.56	0.12
LDH range	4-1960 (426.5±446.4)	4-1000 (228.44±190.40)	U=3.59	<0.001*
Ferritin range	5-970 (453.71±283.36)	7-1500 (278.37±215.35)	U=5.33	<0.001*
D - dimer range	0.06-2150(603.43±519.13)	100-12540 (464.79±882.59)	U=3.51	<0.001*

Table 4: Outcome of cases among cases

Variants	Cases (N = 108)	Control (N = 267)	Test	P value
Survived	107(99.1%)	265(99.3%)	FE	
Died	1(0.9%)	1(0.7%)	0.03	1.0

FE = Fisher's Exact Test

Discussion

Chronic toxoplasmosis is a worldwide parasite with risky or fatal co-infected with other parasites or viruses (Al-Malki, 2021). *Toxoplasma* co-infection with HIV can lead to consequences in immunocompromised persons (Wesołowski *et al,* 2023).

In the present study, anti-*Toxoplasma* IgG was among 28% COVID-19 positive cases. This somewhat agreed with Abdel-Hamed *et al.* (2021) in Egypt, who reported 22.4% *T. gondii* seropositivity among COVID-19 patients and with Geraili *et al*. (2022) in Northern Iran, who found 26.1%, *Toxoplasma* sero-prevalence, but less than Ghaffari *et al.* (2021) also in Iran, who reported a very high seroprevalence of 84%. This discrepancy in prevalence may be attributed to environmental factors, mode of infection, human behavior and pets cats, definitive host (Astrid *et*

al, 2000). In Alexandria, *Toxoplasma* seroprevalence of 22.2% was reported pregnant women (Saleh *et al,* 2014) as compared to 37.4% in Saudi Arabia (Abbas *et al*, 1986) or 47% in Nigeria (Onadeko *et al,* 1992). In Saudi Arabia, even in *Toxoplasma* antibodies were detected in blood donors (Sarwat *et al,* 1993). But, Galván-Ramírez *et al.* (2023) in Mexico reported that latent toxoplasmosis was considered as a risk factor to acquire the COVID-19 in some studies, but others have suggested a negative association between these two infectious diseases.

In the present study, there was decrease in RBC indices in co-infected patients, but neither with significance, nor a correlation with disease outcome. This agreed with Lippi and Plebani (2020) in Italy, who didn't find significant differences as RBCs indices, significant decrease in lymphocytic count or an increase in neutrophil count and suggested that many laboratory parameters ranged in COVID-19 patients. Also, the present results agreed with the hypophysis that COVID-19 patients didn't frequently cause severe anemia (Yang *et al,* 2004). The viral infection of precursor cells and the mature erythrocytes inflammation affected hemoglobin synthesis (Guan *et al,* 2020). Also, Sandri *et al.* (2020) in India, who didn't find any significant disparity in CRP levels, but the elevation in lymphocyte levels and a reduction in neutrophil cells in infected cases, were due to host' trials to regulate infection by various defensive mechanisms. Ali and Saheb (2022) in Iraq didn't find significance of CRP level in COVID-19 patients with or without toxoplaasmosis. Moreover, LDH increased levels, the indicator of pulmonary tissue injury, commonly occurred in COVID-19 patients upon admission to medical facilities (Giuseppe *et al,* 2020).

In the present study, the LDH concentration was significantly elevated in patients as compared to control one. Fan *et al.* (2020) in China reported that individuals with mild infection exhibited LDH values that fall within the reference ranges, in contrast to those in critical condition. Also, Cheng *et al.* (2020) in China reported in LDH alteration occurred in severely patients. Yuan *et al*. (2020) in China reported that serum LDH or CK decline may predict a favorable response to COVID-19 infection treatment. The consistent decrease in the LDH levels may indicate the positive response to the COVID-19 patients' course of infection.

In the present study, the ferritin level was significantly increased in cases group compared to control; in the context of inflammatory conditions, a partial impediment existed in the liberation of iron from the reticuloendothelial system. This agreed with escalated ferritin production pace (Worwood, 1990). The ferritin is the protein responsible for the iron storage, and recognized as a biomarker not only for conditions related to iron metabolism, but also for inflammatory ailments, including diseases characterized by inflammation as a central component, such as cancer, neuro-degeneration, and infectious diseases (Moreira *et al,* 2020).

In the present study, D-dimer was significantly elevated. This agreed with Lippi and Plebani (2020), who declared that the effect of infection on fibrinolysis and alternation of the D-dimer levels occurred in different infection stages. No doubt, the increased evidence as to the pathogenic potential of parasites highlights their ability to affect various physiological parameters within host's body, of which is D-dimer factor that pose a risk for the host in promoting rapid thrombosis and vasoconstriction (Mullarky *et al,* 2006). Abujabal *et al*. (2023) in Saudi Arabia reported that to know the biomarkers predicted the outcome for COVID-19 patients significantly help in the patient's management.

Conclusion

The results showed that changes in the hematological, physiological, and biochemical parameters emphasized the ability of *Toxoplasma gondii* to invade different body cells, especially in immunocompromised hosts.

There was no marked role of toxoplasmosis in the role of COVID-19 infection course.

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Explanation of figure

Fig1: Screening for toxoplasmosis among 375 covid-19 positive patients, 108 patients were seropositive (28.8%)