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IMMUNOMODULATORY EFFECTS OF PARASITIC INFECTION AMONG ONE HUNDRED COVID-19 EGYPTIAN PATIENTS

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Abstract

This study assessed the clinical response to COVID-19 in patients with positive gastrointestinal parasitic infection.

A total number of 100 patients were screened for COVID-19 infection with a nasopharyngeal swab and real-time polymerase chain reaction in the time period from August 2020 till August 2021. Patient's clinical status was classified according to WHO criteria as asymptomatic, mild/moderate, severe and critical. Fresh stool sample specimens were obtained from all patients included in the study for parasites and ova detection. For all cases clinical and laboratory data such as age, gender, results of stool analysis and clinical condition were collected, tabulated and statistically analyzed in corporation with clinical pathology, internal medicine and parasitology departments at Misr University for Science and Technology. This study was conducted on 100 COVID-19 patients whose stool specimens were examined for parasitic infections. The age of the studied patients ranged from 30 to 80 years with 51.21±31.68. They were 44 females and 56 males. The severity of clinical condition among COVID-19 patients was significantly different with the different parasitic infection (P value <0.0001). The 72.2% of asymptomatic COVID-19 patients had positive parasitic infection in their stools, but only 35.5% and 6.1% of the patients suffered from mild and severe clinical conditions respectively, had positive stool results for parasitic infection. The severity of clinical condition among COVID-19 patients didn't significantly different between the protozoa, helminthic or mixed parasitic species.

Key words: COVID-19- Parasitic infection- Severity of clinical condition

Introduction

COVID-19 caused by SARS-CoV-2 virus is the infectious agent responsible for the current pandemic. Since the first cases were identified in Wuhan, China on 2019, the infection spread rapidly, and was declared a pandemic on 2020. Since then, the pandemic has continued unabated, with considerable effects on public health, lifestyle and the global economy (Głuchowska *et al,* 2021). Transmission dynamics of COVID-19 depends chiefly on the respiratory droplets and the direct contact with infected subjects (WHO, 2020). Egypt confirmed its first case of COVID-19 on February 14, 2020, as the first African country had reported the confirmed cases. From February 14, 2020, to April 9, 2021, of the 208,876 laboratory-proved infections, included 12,362 deaths (5.92%) by the SARS-CoV-2 infection, were recorded. According to the official website of the Egyptian MOHP specialized for the news of COVID-19 outbreak in Egypt. Although the incidence and morbidity rates were low, yet it ranks the 7th country in case fatality rate or CFR 5.92% (Saied *et al*, 2021). The viral infectious disease causes severe pneumonia & acute respiratory distress syndrome, a matter that may progress into multi-organ dysfunction and death in a few days to weeks in vulnerable subjects (Zaim *et al,* 2020). Besides, its' effect extended to all organs causing inflammation, vasoconstriction, hypercoagulability and edema, deep venous thrombosis, embolism formation, disseminated intravascular coagulation with ischemic stroke and myocardial infarction (Jain, 2020). However, the less developed countries differ significantly in disease prevalence and conditions from well developed ones. Infectious diseases have a markedly higher prevalence in low developed countries, including the neglected

infectious diseases (Hotez *et al,* 2014). The parasitic infections affect more than 2 billion people throughout the world. Multicellular and highly complex parasites as *Ascaris lumbricoides*, hook worm, *Trichuris* species*, Enterobius vermicularis, Schistosoma* species, *Entamoeba histolytica, Giardia lamblia, Toxoplasma gondii, Cyclospora* and *Cryptosporidium* were among the major cause to the global gastrointestinal parasitosis (Herricks *et al,* 2017). These parasites were encountered nearly all over Egypt (Haridy *et al,* 2009; Youssef and Uga, 2014; Alashry and Morsy, 2021; Shehab *et al,* 2021and others). The T helper type 1 lymphocytes secrete interleukin (IL)-2, interferon-γ, lymphotoxin-α and stimulate type 1 immunity, characterized by intense phagocytic activity. Conversely, T helper type 2 cells secrete IL-4, IL-5, IL-9, IL-10, & IL-13 and stimulate type 2 immunity, characterized by high antibody titers (Spellberg and Edwards, 2001). But, some zoonotic parasites have the capacity to modulate the immune system for their longevity in their hosts (Remuzzi and Remuzzi, 2020). Triggered type-2 responses suppress T helper-1 cells, and expanded populations of Thelper-2 cells and alternatively activated macrophages direct cytokine profiles to IL-4, IL-5, IL-9, & IL-13 (Dearman and Kimber, 2000). Also, helminthes secrete immunomodulatory proteins that skew production of IL-10 in addition to the extension of the regu latory T cell and regulatory B cells, & more inhibition occurred to T helper1 hyper immune activation that characterizes COVID-19 severity (Elliott *et al,* 2007). Helminth-induced alterations in the gut microbes with systemic immunomodulatory action (Brosschot and Reynolds, 2018). This protected from tissue damage by minimizing the inflammatory processes (Mohamed *et al,* 2020).

The study aimed to assess the clinical response to COVID-19 in patients with positive gastrointestinal parasites.

Materials and Methods

After the WHO declaration the COVID-19 became pandemic, the Egyptian Ministry of Health & Population carried out a mass screening of all travelers, people who had come in contact with the COVID-19 patients, health care workers, as well as those with symptoms suggestive of COVID-19. This study included individuals who were enlisted for profiling of COVID-19 infection and subsequently screened for intestinal parasitic infections. A total of 100 patients were screened for COVID-19 infection with a nasopharyngeal swab and real-time polymerase chain reaction (RT-PCR) in the time from August 2020 to August 2021. All patients with confirmed COVID-19 infection ones were admitted to hospital for isolation and treatment, irrespective of their clinical severity status. The symptomatic patients received appropriate therapy according to Egyptian protocol for COVID-19 treatment/management (Saied *et al*, 2021). Asymptomatic patients were quarantined and followed up until discharge. Patient's clinical status was classified according to WHO (2019) criteria as asymptomatic, mild/moderate, severe (with dyspnea, respiratory rate ≥ 30 breaths per minute, O_2 saturation≤ 93%, lung infiltrates≥ 50% of lung fields within 24-48hr), & critical (with respiratory failure, septic shock, and/or multiple organ failure). Severe and critical cases were collected under one category for statistical purposes (Swarnakar and Yadav, 2022). Morning fresh stool samples were obtained from all the included patients for both macro- & microscopic parasites detection. Stool analysis included direct wet mount method, formol ether concentration method and the Modified Ziehl-Neelsen and other indicated stains Garcia, 2007). All the parasites positive patients received the parasite-specific therapy.

Ethical Considerations: The study protocol was approved by the Medical Ethics Committee of MUST University. The approval we nt with the 1975 Ethical Guidelines Declaration of Helsinki (6^{th} Revision, 2008).Written informed consent from the participated patients was obtained after explaining the aim of the study.

Statistical analysis: Data were analyzed by SPSS version 26.0 on IBM compatible computer (SPSS Inc., Chicago, IL, USA). Qualitative data described as number and percentage was analyzed by Chi square test and Fisher's exact test, and tested for normality by Shapiro-Wilks test, with normality at P > 0.05. Quantitative data was described as mean, standard deviation using Student's "t" test, if normally distributed, or Mann-Whitney U test, and Kruskal-Wallis test, if not normally distributed. The P value at < 0.05 was considered significant.

Results

Of Covid-19 patients 39 (39%) showed parasites in stools, and 61 (61%) were negative. Among 39 positive patients, 18 (46%) had protozoa 8 (21%), had helminthes and 13 (33%) had mixed parasites. Ages ranged from 30- 80 (51.21±31.68) years. They were 56 males 7 44 females. Parasite-free Covid-19 patients were older (P<0.0001), without significant difference as to sex and parasites.

Among 100 Covid-19 patients with parasites, 36% were asymptomatic, 31% had mild /moderate clinical pictures and others (33%) had severe clinical conditions, without significant differences (P<0.0001). Ages were more among patients with severe clinical pictures (58.8±12.6) and low among asymptomatic ones (46.9±13.4), without significant difference between sex and severity.

Clinical severity among COVID-19 patients significantly differed as to parasitic infection (P <0.0001). out of 72.2% asymptomatic COVID-19 patients (26/36) had positive stool results for parasitic infection. While only 35.5 % (11/31) and 6.1 % (2/33) of the patients with mild and severe cases respectively, had positive stool results for parasitic infection

Among 18 patients with positive protozoa, 10 (55.6%) were positive for *Entamoeba histolytica* 4 (22.2%) were positive for *Giardia lamblia* 2 (11.1%) were positive for *Blastocystis hominis* and 2 (11.1%) were positive for *Cryptosporidium parvum*. Clinical condition severity among COVID-19 patients didn't significantly differ with protozoa species (P =0.465). Among 8 patients with positive helminthes, 3 (37.5%) had *A. duodenale*, 2 (25%) had *Ascaris lumbricoides,* 2(25%) had *Hymenolepis nana*, and 1 (12.5%) had *E. vermicularis*. Also, clinical condition severity among them didn't signifi cantly differ with species (P =0.659). But, among 13 patients with mixed parasites 3 (23.07%) had *G. lamblia* & *H. nana,* 3 (23.07%) had *G. lamblia* & *E. vermicularis*, 2(15.38%) had *G. lamblia* & *A. lumbricoides*, 1(7.7%) had *G. lamblia* &*A. duodenale,* 2 (15.38%) had *E. histolytica* & *E. vermicularis*, 1(7.7%) had *E. histolytica* & *H. nana,* and 1(7.7%) had *B. hominis* & *A. lumbricoides*. But, the COVID-19 clinical severity among patients was neither significantly differed in mixed parasites (P =0.072), nor in diseases' severity.

Details were given in tables (1, 2, 3, 4, 5, 6 & 7).

Table 1: General characteristics of the studied patients

Variations		Total No. = 100
Age in years	Mean ±SD Median (IQR)	51.21±31.68 49 (39- 64)
Sex	Male no. (%) Female no. (%)	56 (56%) 44 (44%)

Table 2: Relationship between parasitic infection and demographic data among the studied group (n=100)

Variations	Protozoa (n=18)	Helminthes (n=8)	Mixed (n=13)	Negative (n=61)	P value
Age in years	45.7±10.4	43.1±14.9	42.0±10.9	56.1±12.0	<0.0001
Male (n=56)	11 (61.1 %)	4 (50 %)	6 (46.2 %)	35 (57.4 %)	0.834
Female (n=44)	7 (38.9 %)	4 (50 %)	7 (53.8 %)	26 (42.6 %)	

Table 3: Relationship between severity of COVID-19 clinical condition and demographic data (n=100)

Variations	Asymptomatic (n=36)	Mild /moderate (n=31)	Severe /critical (n=33)	P value
Age	46.9 ± 13.4	48.6 ± 12.1	58.8 ± 12.6	<0.0001
Male (n=56)	22 (61.1 %)	17 (54.8 %)	17 (51.5 %)	0.716
Female (n=44)	14 (38.9 %)	14 (45.2 %)	16 (48.5 %)	

Table 4: Relationship between severity of COVID-19 clinical condition and result of stool analysis (n=100)

Clinical picture / Stool analysis	Asymptomatic (n=36)	Mild/moderate (n=31)	Severe/critical (n= 33)	P value
Negative (n=61)	10 (27.8 %)	20 (64.5 %)	31 (93.9 %)	<0.0001
Positive (n=39)	26 (72.2 %)	11 (35.5 %)	2 (6.1 %)	

Table 5: Relationship between severity of COVID-19 clinical condition and positive protozoa (n=18)

Clinical picture / Protozoa	Asymptomatic (n=10)	Mild / moderate (n=7)	Severe/critical (n=1)	P value
Giardia lamblia	2 (20 %)	2 (28.6 %)	00	
Entamoeba histolytica	7 (70 %)	2 (28.6 %)	1 (100 %)	0.465
Blastocystis hominis	00	2 (28.6 %)	00	
Cryptosporidium parvum	1 (10 %)	1 (14.3 %)	00	

Table 6: Relationship between severity of COVID-19 clinical condition and positive helminthes (n=8)

Clinical picture / Helminthes	Asymptomatic (n=5)	Mild/moderate (n=2)	Severe/critical (n=1)	P value
Ascaris lumbricoides	2 (40 %)	00	00	
Enterobius vermicularis	1 (20 %)	00	00	0.659
Hymenolepis nana	1 (20 %)	1 (50 %)	00	
Ancylostoma duodenale	1 (20 %)	1 (50 %)	1 (100 %)	

Table 7: Relationship between severity of COVID-19 clinical condition and mixed parasites among group (n=13)

Clinical picture / Mixed parasites	Asymptomatic (n=11)	Mild/moderate (n=2)	Severe/critical (n=0)	P value
G. lamblia & *H. nana*	3 (27.3 %)	00	00	
G. lamblia & *E. vermicularis*	3 (27.3 %)	00	00	
G. lamblia & *A. lumbricoides*	2 (20 %)	00	00	0.072
G. lamblia & *A. duodenale*	1 (20 %)	00	00	
E. histolytica & *E. vermicularis*	1 (9.1 %)	1 (50 %)	00	
E. histolytica & *H. nana*	1 (9.1 %)	00	00	
Bl. hominis & *A. lumbricoides*	00	1 (50 %)	00	

Discussion

In the present study, laboratory stool and urine examined of 100 COVID-19 PCR confirmed patients didn't show any urinary parasites. However, 39% of them showed gastrointestinal parasites by different stool examination techniques. Among them, 46% suffered from protozoa parasites, and 21% suffered from helminthic ones and 33% showed mixed parasitic infections. The patients mean age was 51.21, the male patients was 56% and the females 44%. The COVID-19 parasite-free patients were significantly of older age than those of parasitic infected ones. Moreover, the mean age was the highest among patients with severe clinical conditions and the lowest was among asymptomatic patients. There was no significant difference between sexes and the parasitic positivity or the COVID-19 severity of clinical manifestations.

Wolday *et al.* (2021) in Ethiopia reported that 37.8% of the COVID-19 patients' harb ored one or more intestinal parasites, protozoa and helminthic infections were 20.2% & 24.5%, respectively. They added that the patients with multiple parasites were 8.7%, with their median ages of 37 years and the majority (63.9%) was male patients. There was no significant difference in sex and age distribution as to parasites infection, but clinical manifestations severity was among the older ones. No doubt, the climatic and ecological conditions in Ethiopia favor the zoonotic parasites abundance

In the present study, the severity of clinical manifestations among the COVID-19 patients showed significant difference between different gastrointestinal parasites. The majority of patients was asymptomatic, but less than half of patients had mild symptoms and minority of them suffered the severe symptoms. This agreed with the (old friends) hypothesis argues that some co-evolved microbes and other pathogens, including helminths helped to establish appropriate immunomodulatory function and thus protecting the host against a large spectrum of immune-

related disorders muting the hyper-inflammation associated with the severe COVID-19 (Rook *et al,* 2004).

In the present study, protozoa among the COVID-19 infected patients were *Entamoeba histolytica, Giardia lamblia* and fewer numbers of *Blastocystis hominis* and *Cryptosporidium parvum*. The severity of clinical condition among COVID-19 patients didn't significantly differ with the different protozoa species. Protozoan parasites such as *Giardia duodenalis* and *Entamoeba histolytica* cause intestinal distress and dysentery (Einarsson *et al,* 2016). Also, *C. parvum*, *C. cayetanensis*, and *T. gondii* are apicomplexan protozoa acquired by ingestion (Dorny *et al,* 2009). But, the parasites can be devastating when they infect immunocompromised persons, and transplacental transmission of *T. gondii* a major cause of congenital birth defects (Morsy *et al,* 2022).

In the present study, patients were positive for *A. duodenale*, *A. lumbricoides, H. nana* and *E. vermicularis* in order of frequency. Severity of clinical condition among Covid-19 patients didn't significantly differ between the helminthic species. Early evidence suggested that the Covid-19 pandemic has had a limited impact on malaria transmission (Roberts, 2020), but two independent analysts predicted that Covid-19-associated disruption of antimalarial drug treatment and insecticide-treated net distribution may lead to a doubling of malaria mortality in Africa (Hillyer, 2020).

In the current study, mixed parasitic infection was in patients (13%), commonest were *G. lamblia* with either *H. nana*, or *E. vermicularis*. The severity of clinical condition among COVID-19 patients didn't significantly differ with mixed infections. But, there was none with mixed parasites among severe critical COVID-19 patients. This agreed with Wolday *et al.* (2021) who reported that patients with multiple parasites gave significant decrease in COVID-19 severity manifestations compared to those without. Also, it agreed with the fact that parasites caused various types of the immunomodulation with changes in the intensity of inflammatory reactions giving the best COVID-19 tolerance (Maizels, 2020).

The cytokine storm seen in severe cases of COVID-19 was characterized by a predominance of pro-inflammatory cytokines, such as IL-6. However, it is possible that parasitic infection could change the outcome of virus infection by modifying the T helper type 2 cells response to limit the inflammatory component (Lucas et *al,* 2021). During parasitic infection, IL-4 and memory CD8+ T cells can increase. Most probably, parasitic infection has forced the human immune response to evolve a safety mechanism based on the induction of highly responsive CD8+ T cells; this would counterbalance the anti-inflammatory effects related to type 2 immunity, with more effective antiviral responses (Fonte et *al,* 2020).

Conclusion

The outcome resulted showed that the parasites whether helminthes or protozoa were associated with less severe symptoms among the COVID-19 patients.

No doubt, the less severities of COVID-19 pathogenicity could be attributed to the parasitic immunomodulation.

Recommendations

1- Patients and families must be educated and encouraged to adhere to social distancing guidelines, use of facemasks and travel guidelines as per CDC guidelines, and social distancing state and local authorities' social distancing protocols.

2- Patients must be educated about frequent hand-washing for a minimum of 20 seconds with soap and water when they come in contact with contaminated surfaces.

3- Patients must be educated and encouraged to seek emergency care when necessary.

References

Alashry, AIA, Morsy, TA, 2021: Zoonotic helminthic nematodes infect human eyes causing from asymptomatic to blindness with reference to Egypt. J. Egypt. Soc. Parasitol. 51, 3:627-40.

Brosschot, TP, Reynolds, LA, 2018: The impa-

ct of a helminthic modified microbiome on host immunity. Mucos. Immunol. 11:1039-46.
Dearman, R, Kimber, I, 2000: Role of $CD4^+$ T helper 2-type cells in cutaneous inflammatory responses induced by fluorescein isothiocyanate. Immunology 101, 4:442-51.
Dorny, P, Prate, N, Dickers, N, Gabriel, S, 2009: Emerging food-borne parasites. Vet. Parasitol. 163:196-206
Einarsson, E, Ma'ayeh, S, Svard, S, 2016: An up-date on *Giardia* and giardiasis. Curr. Opin. Microbiol. 34:47-52
Elliott, DE, Summers, RW, Weinstock, JV, 2007: Helminths as governors of immuno-mediated inflammation. Inter. J. Parasitol. 37:457-64
Fonte, L, Acosta, A, Sarmiento, ME, Ginori, M, García, G, *et al,* 2020: COVID-19 lethality in Sub-Saharan Africa and helminthic immune modulation. Front. Immunol. 11:574910
Garcia, LS, 2007: Intestinal protozoa (Coccidia and Microsporidia) and Algae. In: Diagnostic Medical Parasitology: 5th Ed. American Society for Microbiology.
Głuchowska, K, Dzieci, AT, Edzikowska, SA, Deniziak ZA, Młocicki, D, 2021: The new stat us of parasitic diseases in the COVID-19 Pandemic: Risk factors or protective agents? J. Clin. Med. 10:2533
Haridy, FM, Hassan, AA, Hafez, AO, El-Sherbini, GT, Morsy, TA, 2009: External and intestinal parasites of pet dogs with reference to zoonotic toxocariasis. J. Egypt. Soc. Parasitol. 39, 1:321-6.
Hillyer, JF, 2020: Parasites and Parasitology in this SARS-CoV-2, COVID-19 World: An American Society of Parasitologists Presidential Address. J. Parasitol. 106, 6:859-68.
Herricks, JR, Hotez, PJ, Wanga, V, *et al,* 2017: The global burden of disease study 2013: What does it mean for the NTDs? PloS Negl. Trop. Dis. 11 e0005424
Hotez, PJ, Alvarado, M, Basanez MG, *et al,* 2014: The global burden of disease study 2010: interpretation and implications for the neglected tropical diseases. PloS Negl. Trop. Dis. 8:e2865.
Jain, U, 2020: Effect of COVID-19 on the organs. Cureus 12, 8:e9540.
Lucas. SM, Miguel, GJ, Geller, R, Sanjuan, R, Arévalo, PJ, *et al,* 2021: Potential influence of helminth molecules on the COVID-19 Pathology. Trends Parasitol. 37:11-4**.**
Maizels, RM, 2020: Regulation of immunity and allergy by helminthic parasites. Allergy 75: 524-34
Mohamed, MFH, Mohamed, SF, Yousaf, Z, Kohla, S, Howad F, *et al,* 2020: COVID-19 unfolding filariasis: The first case of SARS-CoV-2 and *Wuchereria bancrofti* co-infection. PloS Negl. Trop. Dis. 14:e0008853
Morsy, TA, Hussein, HE, Morsy, ATA, 2022: TORCH infections, pathogenicity & mortality assessments. JESP 52, 1:53-70.
Remuzzi A, Remuzzi G, 2020: COVID-19 and Italy: What next? Lancet 395, 10231:1225-8
Roberts, L, 2020: Pandemic's fallout on malaria control appears limited so far. Sci. 369: 892-9
Rook, GA, Adams, V, Hunt, J, Palmer, R, Martinelli, R, *et al,* 2004: Mycobacteria and other environmental organisms as immunomodulators for immunoregulatory disorders. Springer Semin. Immunopathol. 25, 3/4:237-55
Saied, AA, Metwally, AA, Abdullah, N, Madkhali, B, Haque S, *et al,* 2021: Egypt's COVID-19 recent happenings and perspectives: A mini-review. Front. Publ. Hlth. 16 August 2021
Shehab, AY, Allam, AF, Farag, HF, Elhadad, H, Kotb, SFA, *et al,* 2021: Intestinal parasites among humans and their livestock animals in a rural community in Gharbia Governorate, Egypt. J. Parasit. Dis. 45, 1:96-100.
Spellberg, B, Edwards, JE, 2001: Type 1/Type 2 immunity in infectious diseases. Clin. Infect. Dis. 32, 1:76-102
Swarnakar, R, Yadav, SL, 2022: Rehabilitation in long COVID-19: A mini-review. World J. Methodol. 12, 4:235-45.
WHO, 2019: Report of WHO-China joint mission on coronavirus disease (COVID-19). Geneva, Switzerland.
WHO, 2020: Modes of Transmission of Virus Causing COVID-19: Implications for infection, prevention and control precaution recommendations: Scientific brief, Geneva, Switzerland.
Wolday, D, Gebrecherkos, T, Arefaine, ZG, Kiros, YK, 2021: Effect of co-infection with intestinal parasites on Covid-19 severity: A prospective observational cohort study. J. Clin. Med. Sep; 39: 101054. doi: 10.1016/j.eclinm. 2021. 101054.
Youssef, AI, Uga, S, 2014: Review of parasitic zoonoses in Egypt. Trop. Med. Hlth. 42, 1:3-14.
Zaim, S, Chong, JH, Sankaranarayanan, V, Harky, A, 2020: COVID-19 and multi-organ response. Curr. Probl. Cardiol. 45, 8:100618.