Original Article

Molecular detection of TNF-α and IL-4 in helminthic and protozoan infected patients in relation to COVID-19 vaccines efficacy in Assiut, Egypt

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ABSTRACT

Background: Newly designed COVID-19 vaccines were approved and administrated worldwide. However, the interaction between parasitic infections and COVID-19 vaccines and their efficacy is still obscure.
Objective: To correlate cytokines (TNF-α and IL-4) levels with COVID-19 vaccine administration in patients with concomitant parasitic infections. A secondary objective is to assess the impact of parasitic infections on COVID-19 efficacy.
Subjects and Methods: The study included 128 patients divided into 2 groups according to an answered questionnaire, and routine laboratory investigations (stool, urine and blood film examination). Both groups included vaccinated (received full doses of COVID-19 vaccine within 6 months of sample collection), and non-vaccinated. A third matching group was recruited as control apparently health participants, *i.e.*, neither parasitic infected nor received COVID-19 vaccines. Molecular detection of cytokines (TNF-α, and IL-4) gene expression was performed for all study samples using real-time PCR.
Results: In comparison to the control group, there was up-regulation of TNF-α in patients with parasitic infection, whether vaccinated or not. According to parasitism, IL-4 showed different gene expression. In case of helminthic infections, it was up regulated in non-vaccinated patients, and down regulated in vaccinated patients. Meanwhile, it was down regulated in patients with protozoal infections whether vaccinated or not.
Conclusion: COVID-19 vaccinated patients with concomitant helminthic infections are susceptible to reduced vaccine efficacy. Generally speaking, parasitism however, could provoke cytokine storm syndrome.

Keywords: COVID-19 vaccine; cytokines; helminths; protozoa.

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INTRODUCTION

Parasitic infections are prevalent worldwide resulting in over one billion people worldwide affected by intestinal parasites[1]. As a result of parasitic infections in humans, the immune system reacts by both humoral immunity and cell mediated immunity[2]. In acute helminthic infections, the patients' immune system responds by an initial Th1 immune response during pre-patency, followed by a Th2-type response during patency. On the other hand, during chronic helminthic infection there is increased production of interleukin-4 (IL-4) and interleukin-10 (IL-10)[3]. Regarding immunity against protozoan infections, TNF- α and IFN-γ are more predominant for the control of parasitemia[4].

The COVID-19 is identified as a strain of severe acute respiratory syndrome related to coronavirus (SARSr-CoV). The WHO reported 516.023 confirmed cases with up to 24,830 by August 2023 in Egypt[5]. From the immunological aspect, the mechanism of COVID-19 virulence is attributed to elevation of inflammatory cytokines (IL-6, IL-10, and TNF- α) which in turn causes depletion of antiviral defenses of innate immunity[6]. Motivated by the extreme morbidities and mortalities of COVID-19, vaccines were developed with up to 95% efficacy and were approved by the FDA, USA[7]. In Egypt, 9 vaccines were approved for immunization including: Oxford/ AstraZeneca, Janssen (Johnson & Johnson), Moderna, Sinovac, Pfizer/BioNTech, Sinopharm (Beijing), Covishield, Gamaleya Sputnik Light and Gamaleya Sputnik. By May 2023, a total of 112,673,535 vaccine doses were administered to the Egyptians[5]. Recent studies reported that vaccination against COVID-19 may result in 'cytokine storm syndrome' in some recipients[6]. In these cases, there is mass release of pro-inflammatory cytokines (IFN-α, IFN-γ, IL-1β, IL-6, IL-12, IL-18, IL-33, TNF-α, TGF-β) and chemokines by the immune effector cells which in turn precipitate and sustain a severe systemic inflammatory response[7]. Additionally, it is believed that as parasitic infections modulate the host immune responses, in turn it could affect vaccination process[8].

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It was reported that COVID-19 vaccines were designed to act by various mechanisms including stimulation of gene expression of TNF-α, and inhibition of IL-4 or not affecting its gene expression[9]. Therefore, both gene expression of TNF-α, and of IL-4 were selected due to their role as significant indicators in parasitic infections and COVID-19 vaccines. We aimed at correlation of cytokines (TNF-α, and IL-4) gene expression in parasitic infected patients with COVID-19 vaccine administration. Moreover, the study aimed to assess the effect of parasitic infections on COVID-19 efficacy.

SUBJECTS AND METHODS

This case-control study was conducted at the Medical Parasitology Department, Faculty of Medicine, Assuit University during the period from October 2021 to May 2024.

Study design: During COVID-19 era and subsequent COVID-19 vaccination campaigns, the study recruited participants attending the outpatient clinics of Assiut city Hospitals. A Questionnaire was designed to collect clinical and demographic data, and according to laboratory investigations, participants were equally divided into 3 groups. Participants included patients with parasitic infection either vaccinated or not. The third group included control healthy individuals. Cytokines (TNF-α, and IL-4) gene expression were molecularly determined to correlate their levels with vaccine administration, and efficacy.

Study population: The study recruited adults of both genders with age limit between 20-60 y who attended Assiut city hospitals during COVID-19 vaccination. Inclusion criteria for the study groups were vaccinated and non-vaccinated participants with COVID-19 vaccines who were proved to have different parasitic infections (helminthic and protozoan), and vaccinated cases who must have completed all doses of vaccination within 6 months). Exclusion criteria included patients with any diseases or infections, other than parasites, that affect cytokines levels (mainly IL-4, TNF-α). On the other hand, the control group included apparently healthy individuals who were not vaccinated. They were either healthy relatives of coming participants, or healthy individuals enrolled from blood donation bank in Assuit city hospital. Either way their samples were examined to confirm absence of parasitic infections.

Study questionnaire: A Questionnaire was designed to collect data covering: name, age, sex, residency, occupation, symptoms of parasitic infections, COVID-19 vaccine administration or not, the type of vaccine taken, time of last dose taken.

Samples collection: Stool and urine samples were collected in dry, clean, leak-proof plastic disposable cups. Blood samples (~2ml) were withdrawn in sterile EDTA tubes. All samples were labeled with name, age, date and sex.

Samples examination: Stool samples were examined macroscopically for consistency, color, odor, and presence of blood and/or mucus, microscopically using saline and iodine direct wet mount, formalin-ethyl acetate sedimentation concentration method and modified Kinyoun's acid fast stain (cold) to confirm the diagnosis of different parasites[10,11]. Urine samples were examined macroscopically for turbidity, hematuria and color change and microscopically using sedimentation and filtration methods for detection of parasitic infections; *Schistosoma* spp., and *T. vaginalis*[11]. Blood examination was performed grossly by sample inspection to exclude clotting or hemolyzed samples. Microscopically, a Giemsa-stained thin blood film was performed to detect blood parasites[11].

Molecular detection of cytokines gene expression using quantitative real time PCR (qRT-PCR)[12]

1. **Extraction of RNA:** Total RNA was extracted *via* the ABT Total RNA Mini Extraction kit (Applied Biotechnology Corporation, Egypt, catalog No. ABT001). Briefly, RNA isolation was performed in an RNase-free environment and all steps were performed at room temperature. Blood samples were lysed and homogenized using Trizol reagent in conjunction with the extraction kit. Since the kit had a unique RNA lysis buffer that rapidly lysed cells and inactivates cellular nuclease, RNA was selectively adsorbed to silicified membrane. Cellular metabolite, proteins and inhibitors were removed by washing with potent washing buffers. Finally, the purified RNA was eluted from silica membrane by low salt elution buffer.

2. **Reverse transcription (cDNA synthesis):** The complementary DNA (cDNA) synthesis kit (ABT H-minus cDNA Synthesis Kit, Applied Biotechnology Corporation, Egypt, catalog No. ABT009) was used to obtain the cDNA samples needed for qRT-PCR According to manufacturer's instructions, reactions were run on a thermal cycler (Veriti® 96-Well, The Applied Biosystems®, USA).

3. **The qRT-PCR reaction** was carried out according to manufacturer's instructions using the QuantStudio™ 5 RT-PCR instrument (96-well 0.2 ml block). Thermal profile was performed in steps: one cycle of initial denaturation for 3 min at 95°C; 45 cycles of denaturation at 95°C for 15 sec; annealing for 30 sec at 50-60°C; extension for 30 sec at 72°C. Sequences of the primers used for qRT-PCR reaction were TNF-α (Sense: CCCCAGGGACCTCTCTCTAATC, Antisense: GGTTTGCTACAACATGGGCTACA), and IL-4 (Sense: AACAGCCTCACAGAGCAGAAGAC, antisense: GCCCTGCAGAAGGTTTCCTT). Besides, we used β-actin (Reference gene) Sense:

GGATGCAGAAGGAGATCACTG, Antisense: CGATCCACACGGAGTACTTG)[13].

4. **Results interpretation:** At the end of the reactions, results were analyzed using QuantStudio™ Design & Analysis Software v1.5.3 [comparative cycle threshold (Ct) method][14]. This method is a mathematical model that calculates changes in gene expression as a relative fold difference between the healthy control group and both vaccinated and non-vaccinated parasitic infected groups.

Statistical analysis: Data entry and cleaning were done using Excel program to be converted to SPSS Statistics (version 26.0, IBM) for analysis. Data were checked for accuracy and normality using Sharpiro-Wilk tests. Statistical analysis utilized X^2, Mann Whitney U, and Kruskal Wallis tests to compare between quantitative variables. The *P* value was considered significant when equal to or less than 0.05.

Ethics statement: Written informed consent was obtained from all participants before enrollment with explanation of the study aim. All participants were informed with the results of their laboratory investigations. Infected patients were informed in order to seek treatment. The research protocol was approved by the ethics committee of the Faculty of Medicine, Assiut University (IRB local approval number:17101704).

RESULTS

Demographic data: As described in table (1), participants' age range was 20-60 y. The mean age of parasitic infected vaccinated and non-vaccinated groups was 37.53±11.53, and 30.17±10.75 respectively, while it was 40.94±10.54 among controls. The percentage of females infected with different parasites was higher than males.

Table 1. Demographic characteristics of the studied participants.

Parameter	History of COVD-19 vaccine administration in parasitic infected patients		Control (N=64)	Total (N=192)
	Vaccinated (N=64)	Non-vaccinated (N=64)		
Age (years)				
Mean±SD	37.53±11.53	30.17±10.75	40.94±10.54	36.21±11.78
Range	20-60	20-60	21-60	20-60
Gender				
Male	20 (31.2%)	27 (42.2%)	33 (51.6%)	80 (41.7%)
Female	44 (68.8%)	37 (57.8%)	31 (48.4%)	112 (58.3%)
Residence				
Rural	38 (59.4%)	32 (50.0%)	32 (50.0%)	102 (53.1%)
Urban	26 (40.6%)	32 (50.0%)	32 (50.0%)	90 (46.9%)

Parasitological examination: The study detected the following parasites: *S. heamatobium, S. mansoni, E. vermicularis, C. philippinensis, Cryptosporidium* spp., *E. histolytica/dispar, Blastocystis* spp., *G. lamblia* and *Plasmodium* spp.

Gene expression of cytokines: In helminthic infections, the gene expression level of TNF-α in vaccinated group was higher than non-vaccinated group. Both groups were higher than controls (*i.e.*, up regulation). The difference was statistically significant ($P<0.05$). On the other hand, the gene expression level of IL-4 in vaccinated helminthic infections was lower than controls (*i.e.*, down regulation). While in non-vaccinated helminthic infections, IL-4 level was higher than controls (*i.e.*, up regulation) with statistically significant difference ($P<0.01$) (Table 2, and Fig. 1).

In protozoan infected patients, gene expression level of TNF-α was higher in vaccinated group than non-vaccinated group, and both groups were higher than controls (*i.e.*, up regulation). The difference was statistically significant ($P<0.05$). The gene expression level of IL-4 in both groups (1 and 2) was lower than controls (*i.e.*, down regulation). However, IL-4 gene expression level was slightly higher in non-vaccinated group (2) than vaccinated group (1). The difference between groups was not statistically significant (Table 3, and Fig. 2).

Table 2. Detection of TNF-α and IL-4 gene expression level in helminthic infections and its correlation to COVID-19 vaccine administration.

Cytokine gene expression	Healthy control (N=64)	Helminthic infections		Statistical analysis
		Vaccinated (N=18)	Non-vaccinated (N=18)	
TNF-α (Mean±SD)	1.00±3.94	3.59±1.92	2.31±2.71	$P<0.05$*
IL-4 (Mean±SD)	1.00±1.21	0.48±1.29	2.35±1.79	$P<0.01$*

Kruskal-Wallis test was used. ***:** Significant ($P<0.05$).

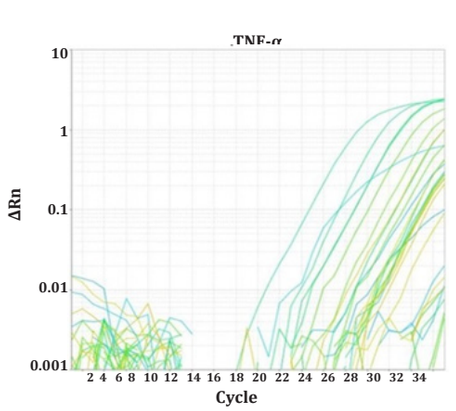

Fig. 1. Amplification plot of TNF-α as obtained from the used software.

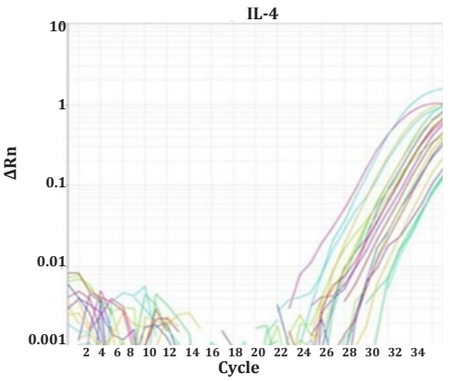

Fig. 2. Amplification plot of IL-4 as obtained from the used software.

Table 3. Detection of TNF-α and IL-4 gene expression level in protozoan infections and its correlation to COVID-19 vaccine administration.

Cytokine gene expression	Healthy control (N=64)	Protozoan infections		Statistical analysis
		Vaccinated (N=46)	Non-vaccinated (N=46)	
TNF-α (Mean±SD)	1.00±3.76	4.84±1.90	3.69±1.99	*P*<0.05*
IL-4 (Mean±SD)	1.00±3.57	0.236±2.40	0.264±3.41	*P*<0.01*

Kruskal-Wallis test was used. ***:** Significant (*P*<0.05).

DISCUSSION

Demographic data of the study groups revealed that the age with highest infection rates was between 30 and 37 y. This could be attributed to the fact that middle age is the peak of personnel activity with more exposure to the environment that made them more susceptible to parasitic infections. Similar results were obtained in Iran[15] and Emirates[16]. Infections were more evident among females rather than males which can be explained by the lifestyle of Egyptian females, and food preparation with possibility of exposure to parasitic eggs or cysts contaminating water and food. These findings were compatible with earlier studies conducted in Nepal[17] and Somalia[18]. Moreover, the majority of the study participants were from rural residence. The main reasons were mostly rural poor hygienic condition, lower educational status, poverty and low socioeconomic status compared to urban ones[19]. Similarly, intestinal parasitic infections were significantly higher in rural versus urban areas in Ethiopia[20], Iran[21], Nepal[17], and Egypt[22].

The study revealed that some cases developed post-vaccination COVID-19 infection which can be explained by the fact that parasites cause systemic immunomodulatory effects due to alteration in the gut microbiome leading to suppression of host immune responses, which in turn affects vaccine efficacy[23]. In addition, chronic helminthic infections were recorded to stimulate Th2 immune response which in turn causes suppression of the immune response against intracellular pathogens rendering the host susceptible to infections as corona virus[24]. This was also observed in a study conducted in Qena, Egypt[25].

Moreover, highly sensitive based quantitative real time PCR showed statistically significant increase (up-regulation) of TNF-α gene expression in parasitic infected patients by either helminths or protozoa. However, the mean fold change of TNF-α gene expression level in the vaccinated group was higher than non-vaccinated group. We hypothesized that the expression pattern of TNF-α might be increased due to the co-existence of both factors, *i.e.*, parasitism and vaccination. This in turn could augment immune response in susceptible individuals leading to serious complication as "cytokine storm syndrome". Similarly, it was confirmed that there was an increase in TNF-α gene expression level in response to various helminthic and protozoan infections[1]. Meanwhile, it was claimed that COVID-19 vaccines administration was accompanied with an increase in TNF-α gene expression level as a part of Th1 polarization[9].

On another scale, IL-4 gene expression level showed statistical significance in helminthic infected patients as there was up-regulation in non-vaccinated group and down- regulation in those vaccinated. This can be explained by the fact that helminthic infections trigger Th2 action with production of IL-4 to compete the infection. However, COVID-19 vaccines administration triggers Th1 cells mainly, and subsequently, there were two opposing actions of Th1 and Th2. However,

Th1 seemed to predominate with down regulation of IL-4 gene expression level. This in turn could worsen the helminthic infection outcome leading to different complications.

Regarding protozoan infected patients, there was (down-regulation) in IL-4 gene expression in both groups with no statistical significance. This can be justified by the fact that protozoan infections trigger Th1 cells which counteract Th2 cells leading to their suppression with low IL-4 gene expression level. Besides, IL-4 gene expression level was slightly higher in non-vaccinated group than vaccinated group as COVID-19 vaccines trigger Th1 action that oppose Th2 as well. As a result, there was more decrease of IL-4 gene expression level in vaccinated protozoan infected participants. Two studies[2,26] were in line with the present study regarding the role of Th2 and its secreted cytokines as IL-4 in helminthic infections. Additionally, previous studies ensured the role of Th1 in competing protozoan infections[3,27].

Vaccine efficacy is questionable in parasitic infected candidates. This study revealed post vaccination COVID-19 infection in helminthic infected cases. This is attributed to altered immune response in these patients which interact with vaccine induced immunity leading to attenuated vaccine response[28]. On another aspect, the overproduction of TNF-α as a response to parasitic infections and COVID-19 vaccines was found to increase risk of cytokine storm syndrome. This syndrome was described as a hyper-inflammatory state secondary to the excessive production of large amounts of proinflammatory cytokines including TNFα. This exaggerated response can lead to fatal complications to the host ranging from acute respiratory distress syndrome, coagulopathy, multi-organ failure and even death[6].

In conclusion, parasitic infections co-presence in COVID-19 vaccinated candidates can lead to reduced vaccine efficacy. Cytokine storm syndrome could be provoked in COVID-19 vaccinated susceptible parasitic infected individuals leading to serious complications up to being fatal. Screening and treatment of parasitic infections is recommended before COVID-19 vaccines administration to eliminate parasites suppressive impacts on vaccination effectiveness. For the fear of provoking cytokine storm syndrome, we recommend examination of samples from suspected parasitic infected individuals, especially in parasitic endemic countries as Egypt, to avoid post vaccination cytokine storm syndrome.

Moreover, first dose vaccinated candidates should be monitored for any side effects before receiving the second dose to assure safe vaccine administration and avoid induced complications as thrombocytopenia and different immunological responses. Eventually, further studies are recommended to reduce COVID-19 vaccines induced side effects.

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REFERENCES

1. Donnelly S. The immunology of parasite infections: Grand challenges. Front Parasitol 2022; Available online at https://www.frontiersin.org/journals/parasitology/articles/10.3389/fpara.2022.1069205/full.
2. Rolot M, Dougall M, 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.
3. Silva-Barrios S, Stäger S. Protozoan parasites and type I IFNs. Front Immunol 2017; 19(1):8-14.
4. Elsaftawy E, Wassef R, Amin N. Can endemic parasitic diseases and/or vectors play a role in the COVID-19 pandemic. PUJ 2021; 14(1): 7–14.
5. WHO. Weekly epidemiological update on COVID-19.2023; Available online at: https://www.who.int/publications/m/item/weekly-epidemiological-update-on- covid-19-1-September-2023.
6. Costela-ruiz J, Illescas-montes R, Puerta-puerta M, Ruiz C, Melguizo- rodríguez L. The role of cytokines in COVID-19 disease. Cytokine Growth Factor Rev 2020; 54:62-75.
7. Farshi E. Cytokine Storm Response to COVID-19 Vaccinations . J Cytokine Biol 2020; 5(2):34.
8. Zakeri A, Hansen EP, Andersen SD, Williams AR, Nejsum P. Immunomodulation by helminths: intracellular pathways and extracellular vesicles. Front Immunol 2018;9:2349.
9. Sadarangani M, Marchant A, Kollmann T. Immunological mechanisms of vaccine-induced protection against COVID-19 in humans. Nat Rev Immunol 2021; 21:475–484.
10. Methanitikorn R, Sukontason K, Sukontason KL, Piangjai S. Evaluation of the formalin-Tween concentration technique for parasitic detection. Rev Inst Med Trop Sao Paulo 2003;45(5):289-291.
11. Garcia LS. Diagnostic Medical Parasitology. ASM, 2016; 6th Edition. Available online at: https://doi.org/10.1002/9781119021872.ch15.
12. Samadikuchaksaraei A. Polymerase Chain Reaction for Biomedical Applications. InTech; 2016. Available from: http://dx.doi.org/10.5772/62968.

13. Boeuf P, Vigan-Womas I, Jublot D, Loizon S, Barale J, Akanmori B. *et al*.. CyProQuant-PCR: A real time RT-PCR technique for profiling human cytokines, based on external RNA standards, readily automatable for clinical use. BMC Immunology 2005; 6:5.
14. Livak J, Schmittgen D. Analysis of relative gene expression data using real- time quantitative PCR and the 2(-Delta Delta CT) method. Methods; 2001; 25(4):402-408.
15. Heidarii F, Akhondimeybodi Z, Sarcheshme A, Hamidfar M, Shahcheraghi S, Mousavi S *et al*. The prevalence of intestinal parasitic infections among applicants for receiving the health card. J Med Res 2021; 7(4):102–104.
16. Al-Rifai H, Loney T, Sheek-Hussein M, Zoughbor S, Ajab S, Olanda M *et al*. Prevalence of and factors associated with intestinal parasites in multinational expatriate workers in Al Ain city, United Arab Emirates: An occupational cross-sectional study. J Immigr Minor Health 2020; 22(2):359–374.
17. Bertoncello C, Amoruso I, Moscardino U, Fonzo M, Maharjan M, Buja A, *et al*. Sex-biased prevalence of intestinal parasitic infections and gender inequality in rural Nepal. IJID 2021; 109:148–154.
18. Doğan S, Mohamud M, Mohamud H, Ali M, Orhan Z. Distribution of the intestinal parasites according to species and gender in patients presented to the Microbiology laboratory in a Tertiary Hospital, in Somalia between January 2018 and October 2022. Infect Drug Resist 2023; 16:7007–7014.
19. Karim A, Zartashia B, Khwaja S, Akhter A, Raza A, Parveen S. Prevalence and risk factors associated with human intestinal parasitic infections (IPIs) in rural and urban areas of Quetta, Pakistan. Braz J Biol 2024; 84: 1–5.
20. Workneh T. Prevalence of intestinal parasitic infections and associated factors among Debre Elias Primary Schools Children, East Gojjam Zone, Amhara Region, North West Ethiopia. J Bacteriol Parasitol 2014; 05(01):1–5.
21. Montresor A, Mupfasoni D, Mikhailov A, Mwinzi P, Lucianez A, Jamsheed M *et al*. The global progress of soil-transmitted helminthiases control in 2020 and World Health Organization targets for 2030. PLoS NTDs 2020; 8(8):1–8.
22. Ahmed M, Abu-Sheishaa A. Intestinal parasitic infection among school children in Dakahlia governorate, Egypt: a cross-sectional study. Gaz Egypt Paediatr Assoc 2022; 70(1):4–11.
23. Abdoli A. Helminths and COVID-19 co-infections: A neglected critical challenge [Article-commentary]. ACS Pharmacol Transl Sci 2020; 3(5):1039–1041.
24. Fonte L, Acosta A, Sarmiento E, Ginori M, García G. COVID-19 lethality in Sub-Saharan Africa and helminth immune modulation. Front Immunol 2020; 11:10–12.
25. Mohamed M, Awadein M, El-Taweel A. Immunomodulatory effects of parasitic infection among one hundred COVID-19 Egyptian patients. JESP 2022; 52(3):389–394.
26. Gazzinelli-Guimaraes PH, Nutman TB. Helminth parasites and immune regulation. F1000Res 2018; 23:7.
27. Engwerda C, Ng S, Bunn T. The regulation of $CD4^+$ T cell responses during protozoan infections. Front Immunol 2014; 1–9.
28. Chacin-Bonilla L, Chacón-Fonseca N, Rodriguez-Morales J. Emerging issues in COVID-19 vaccination in tropical areas: Impact of the immune response against helminths in endemic areas. Trop Med Infect Dis 2021; 42:102087.