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ORIGINAL PAPER

Serum levels of IFN-γ and IL-4 in hospitalised COVID-19 patients – evidence of reduced cytokine storm in discharged patients

Ganiyu Olatunbosun Arinola [1], Abdulfattah Adekunle Onifade [1], Kehinde Adigun [2], Mosunmade Babatunde Oshingbesan [3]

[1] Department of Immunology, University of Ibadan, Nigeria
[2] Department of Family Planning, University College Hospital, Ibadan, Nigeria
[3] Independent Researcher, Ipswich, United Kingdom

ABSTRACT

Introduction and aim. Coronavirus disease 2019 is characterised by cytokine storm and it was managed with repurposed drugs, however the effect of this treatment on cytokine storm is unknown. The aim of the study was to investigate the effect of repurposed management on serum Th1 pro-inflammation cytokine (IFN-γ) and Th2 anti-inflammation cytokine (IL-4) in COVID-19 patients.

Material and methods. The levels of IFN-γ and IL-4 were determined in sera from 45 COVID-19 patients at admission followed-up till discharge after repurposed treatment using ELISA. The mean levels and proportions above normal reference ranges of IFN-γ and IL-4 were compared in COVID-19 at admission and discharge.

Results. The mean values of IFN-γ and IL-4 were significantly higher in COVID-19 patients at admission compared with discharged COVID-19 patients whereas IFN-γ:IL-4 ratio was significantly higher in discharged COVID-19 patients compared with admitted COVID-19 patients. Significantly higher proportion of COVID-19 patients at discharge had IFN-γ within the normal reference ranges compared with COVID-19 patients at admission whereas the proportions of COVID-19 patients at discharge and COVID-19 patients at admission having IL-4 within the normal reference ranges were the same.

Conclusion. Cytokine storm was evidenced in COVID-19 patients at admission and repurposed treatment suppressed pro-inflammation cytokine (IFN-γ) in most discharged COVID-19 patients.

Keywords. cytokine storm, re-purposed treatment, SARS-CoV-2

Introduction

The coronavirus disease 2019 (COVID-19) pandemic, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has been a global disaster since December 2019.[1] As at July 08, 2023, there was 266,675 COVID-19 cases, 3155 death by COVID-19 and 259,953 patients recovered.[2] The mortality rates caused by COVID-19 in different regions of the world varied with demographic variables, presence or absence of comorbidities, immune status, exposure to malaria parasites or use of anti-malaria drugs, blood group and lifestyle.[1,3-7] COVID-19 severity have been associated with hyperinflammation caused by elevated levels of proinflammatory cytokines such as interleukin (IL)-6, nitric oxide (NO), C-reactive protein (CRP), deranged blood cell counts and lymphopenia.[6-11]

SARS-CoV-2 exhibits clinical characteristics ranging from asymptomatic to severe acute respiratory dis-

Corresponding author: Ganiyu Olatunbosun Arinola, e-mail: drarinolaog64@yahoo.com

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tress syndrome, septic shock, and/or multiple organ dysfunctions, leading to death.[12,13] Specific treatment for COVID-19 is not known but many therapeutic strategies involved the use of anti-inflammatory-, antiprotozoan-, antioxidant- agents, antibiotics, moderate exercises and protein rich diets.[13-16] This study predicted that repurposed treatment adopted in our IDC would reduce the cytokine storm and improve the clinical indices of patients with COVID-19. However, because of limited resources and unavailability of data from hospitalised patients at admission and discharge in Nigeria, it was difficult to ascertain the effectivenss of prescribed repurposed treatment (a process of identifying new therapeutic use(s) for old/existing/available drugs) in our setting. Also, because of our concern about the disproportionate immunopathologic burden of COVID-19, we aimed to elucidate the Th1-Th2 cytokine parameters in admitted and discharged patients with COVID-19, which can help distinguish immune signature or explain immune regulation leading to determining better COVID-19 treatment. In addition, a study to ascertain proportions of COVID-19 patients in Nigeria having cytokine levels above normal ranges is yet to be performed. In line with that, this study analysed proportion of individuals with COVID-19 having cytokine levels above normal ranges.

Immunological biomarkers have been suggested as primary drivers of morbidity and mortality with COVID-19. Several cytokines have been correlated with COVID-19 severity. Most notably, elevated IL-4 and IL-6 while reduced IFN-γ were detected in hospitalized patients, especially critically ill patients.[17] Increased IL-2R, IL-8, IL-10, and GM-CSF have been associated with disease severity but studies are limited.[18] Conflicting results regarding IL-1β and IL-4 were reported.[19] Elevated cytokine concentrations of IL-6, IL-10, IL-18 and IFN-γ have been widely described in COVID-19 patients but these did not seem to have prognostic values.[17] IL-4 was associated with impaired lung lesions, but some reports point to a potential mediator effect.[17-19]

Cytokines that enhances inflammatory responses(pro-inflammatory cytokines) include tumor growth factor-beta (TGF-β), gamma interferon (IFN- γ), IL-1, IL-8, IL-6, TNF-α, while some other cytokines function to reduce the inflammation (anti-inflammatory cytokines) like IL-4 and IL-10.[7] Therefore, it becomes important to analyse the cytokine load (IFN-γ and IL-4) in the sera of patients with COVID-19 in comparison to reference normal ranges and treatment success or effectiveness.

Aim

In this longitudinal study, the serum levels of IFN-γ and IL-4 were measured through ELISA in sera from patients with COVID-19 at admission and discharge compared with normal reference ranges in order to decipher the cellular mechanism behind the treatment of coronavirus. To our knowledge, this is the first report regarding the effectiveness of repurposed treatment using cytokine release in COVID-19 patients.

Material and methods

Ethical approval was granted by the Institutional Ethical Committee with approval number UI/EC/20/0283. The participants comprised of 45 laboratory confirmed COVID-19 patients at admission followed up till discharge. The study was conducted between 6th May, 2020 and 19th July, 2020. None of the patients was in severe or critical condition and were able to give consent.The duration between admission and discharge of COVID-19 patients was 3–10 days. The participants did not have hypertension, diabetes mellitus, cardiovascular disease, cerebrovascular disease, cancer, chronic renal disease or inflammatory conditions. Serum was removed from clotted whole blood collected in a test tube without anticoagulant by centrifuging at 1500×g for 10 minutes. Interferon-gamma and IL-4 levels in the serum were determined using ELISA as previously described using manufacturer's instruction.[11] The values of IFN-γ and IL-4 obtained were compared with the reference value of 0.1–2.8 pg/L for serum IL-4 and reference value of 0.16-7.42 pg/L for serum IFN-γ to determine the proportion of patients having IFN-γ or IL-4 values above reference ranges.[20] Data from this comparison were represented as frequencies and percentages. Chi-square test was used to determine the differences between the frequencies while the differences in the mean of variables of COVID-19 patients at admission and discharge were compared using Student t-test. $p \leq 0.05$ was considered as statistically significant. A treatment protocol designed by the Case Management Team of the Oyo State COVID-19 Task Force was adopted.[13] The protocol included treating with a cocktail of chloroquine or hydroxychloroquine, zinc, vitamins C and D and or antibiotic(s) as indicated. Physiotherapy and nutritional support for these patients were also considered as priority.

Results

Vitamin C, vitamin D and Zn were given to COVID-19 patients from day of admission for 3 weeks while azithromycin and chloroquine or hydroxylchloroquine were given for 3 days (Table 1). The mean values of IFN-γ and IL-4 were significantly higher in COVID-19 patients at admission compared with COVID-19 patients at discharge whereas the mean value of IFN-γ:IL-4 ratio was significantly higher in COVID-19 patients at discharge compared with COVID-19 patients at admission (Table 2). Significantly higher proportion of COVID-19 patients at discharge had IFN-γ within the normal ref-

erence ranges compared with COVID-19 patients at admission whereas the proportions of COVID-19 patients at discharge and COVID-19 patients at admission having IL-4 within the normal reference ranges were the same (Table 3).

Table 1. Medication used among COVID-19 patients at IDC, Ibadan, Nigeria

Medications	COVID-19 Patients
Vitamin D (1,000 IU)	1,000 iu twice daily for 3 weeks
Vitamin C (1,000 mg)	1,000 mg twice daily for 3 weeks
Zn (20 mg)	100 mg daily for 3 weeks
Azithromycin	500 mg daily for 3 days
Hydroxychloroquine	400 mg on day 1 and 200 mg daily for 3 more days
Chloroquine (as an alternative to hydroxychloroquine)	500 mg on day 1 and 250 mg daily for 3 more days

Table 2. Mean (±SD) values of IFN-γ and IL-4 in COVID-19 patients at admission compared with COVID-19 patients at discharge

Variable	COVID-19 patients at admission (n=45)	COVID-19 patients at discharge (n=45)	p
IFN-γ (pg/L)	0.162±0.96	0.149±0.099	<0.05
IL-4 (pg/L)	0.3±0.1	0.1±0.1	<0.05
IFN-γ/IL-4 ratio	0.51±0.03	1.23±0.98	<0.05

Table 3. Frequency (percentage) of COVID-19 patients at admission compared with COVID-19 patients at discharge having of IFN-γ and IL-4 within and outside reference ranges*

Variable	Category	COVID-19 patients at admission (n, %)	COVID-19 patients at discharge (n, %)	p
IFN-γ	Within RR	31 (70.45)	33 (75)	<0.05
	Outside RR	13 (29.55)	11 (25)	
IL-4	Within RR	43 (100)	43 (100)	>0.999
	Outside RR	0 (0)	0 (0)	

* RR – reference range

Discussion

COVID-19 patients develop clinical signs ranging from mild to life-threating symptoms as a result of SARS-CoV-2 invasion of epithelial cells of the mucosal surfaces through attachment to angiotensin-converting enzyme 2 (ACE2) receptor.[18] In the cytosol of lung cell, double stranded RNA replication stimulates hyperinflammation and the presence of ACE 2 receptors on several organs in humans apart from lungs, causes multi-organ damages by SARS-CoV-2.[12] However, the present study proposed that excessive stimulation of cytokine production might be responsible for organ damage in COVID-19 patients, which was curtailed by repurposed drugs. Understanding the precise drivers of immune dysfunction is crucial to guide the application of appropriate immunomodulatory treatments since management strategies of COVID-19 are not specific. Previous management strategy of COVID-19 patients involved the use of immunosuppressive therapies, anti-inflammatory and immunomodulatory agents.[13-16] Due to variations in the management strategies of COVID-19, the mechanism of individual repurposed treatment might vary. The present study suggests that reduction of cytokine storm might be one of the actions mitigated by repurposed management strategies applied in our IDC.

There have been several studies comparing cytokine levels in COVID-19 patients and control but a longitudinal study (comparing admitted versus discharged patients) or comparing data with normal reference ranges will give better clinical relevant information.[17-20] Moreover, inconsistent results were obtained for cytokine levels in COVID-19 cases versus unifected control studies. No study till date, have reported prevalence of COVID-19 patients having biological markers within and outside normal reference ranges at admission or during treatment. IFN-γ is an antimicrobial protein upregulated in the COVID-19 patients.[17,18] Our result of raised mean IFN-γ level in newly admitted COVID-19 patients is supported in a study by Zhou et al., which reported a significantly increased peripheral blood monocytic cells frequency of polyclonal GM-CSF+CD4 T cells capable of prodigious ex-vivo IFN-γ production only in critically ill COVID-19 patients.[21] IFN-γ-mediated Th1 plays a critical role in antigen-specific defense mechanisms at the epithelial surface associated with multiple inflammatory airway diseases.[17] Other reports stated that IFN-γ acts as a regulator of efficient antigen presentation, and lowering the IFN-γ level or deviation in the IFN-γ-IFN-γR system severely impedes host immune responses to infections.[22] A study also showed that IFN-γ level was associated with respiratory distress and mortality in patients with COVID-19.[23] Other studies showed that IFN-γ induces PANoptosis and a deadly cytokine storm, causing acute lung damage and mortality among patients.[24] It is likely that the natural defence phenomenon of the host was to stimulate concurrent rise in IL-4 level to conteract this negative effect of raised IFN-γ level during SARS-CoV-2 infection.

Limited data existed for IL-4 level in COVID-19 patients, though IL-4 was found to be associated with impaired lung lesions and its potential mediator effect was emphasised in COVID-19 patients.[25] A data reported that IL-4 level was significantly increased, whereas the level of IFN-γ was significantly reduced in patients with severe COVID-19 compared with those in patients with mild and/or moderate COVID-19.[17] Increase in IL-4 level, but no reduction in the IFN-γ level has also been reported by other study.[26] High levels of IFN-γ and IL-4 observed in newly admitted COVID-19 patients

compared with discharged COVID-19 implied cytokine storm in COVID-19 patients and this was reported contributing to tissue damage in the respiratory tract and in other organs.[7,12,18,19] Also, reduced IFN-γ and IL-4 in discharged COVID-19 compared with COVID-19 at admission could also be linked with the fact that adaptive immunity (both humoral- and cellular mediated responses but more especially cellular mediated response) is needed for recovery of COVID-19 patients. In addition, our data suggest that the systemic inflammation in COVID-19 patients at admission was due to ongoing infection and viral replication as confirmed by absence of SARS-CoV-2 by PCR analysis in all discharged patients after repurposed therapy. The implication of our finding is that repurposed treatment suppressed systemic inflammation.

The cytokine environment determines the subsets of predominant T helper cells by influencing their differentiation. IFN-γ is critical for determining Th1 development, whereas IL-4 is necessary for the development of Th2 proliferation, thus Th1-Th2 balance determines the disease outcomes through regulation of cellular immune responses and inflammation.[27] It has also been reported that higher IFN-γ:IL-4 ratio (higher Th1/Th2) mediate diminished serum antibodies and increased production of cell-mediated immunity.[27] Among intriguing finding of our study is that discharged COVID-19 patients have a higher IFN-γ:IL-4 ratio than in newly admitted COVID-19 patients. This implied an enhanced cell-mediated immunity in discharged COVID-19 patients. This supported previous findings of increased lymphocyte numbers in recovered patients but decreased number in non-surviving patients.[18-20] Our present finding of higher IFN-γ:IL-4 ratio in discharged COVID-19 patients compared with newly admitted COVID-19 patients also supported the established fact that cell-mediated immunity is needed for the control of viral infection and that lethal outcomes in virus infection correlated with elevated Th2 cell serum cytokines including IL-4.[22-25,28]

Vitamin intake lowers oxidative stress markers, alleviates cytokine storm, reduces disease severity, lowers-proinflammatory cytokine, -hyperinflammation and -organ failure.[29] Vitamin C as a basic exogenous vitamin is known for its strong antioxidant and anti-inflammatory properties because it scavengers free radical, activates and stimulates biosynthesis of antioxidants. Other involvement of vitamin C against inflammation encompasses modulation of nuclear transcription factor kappa B (NF-kB) and lowering of pro-inflammatory cytokines production.[30] An observation corroborated the anti-inflammatory and immune supporting properties of vitamin D which usually result in dampening of hyperinflammation.[31] Zinc supplementation decreases -oxidative stress biomarkers, -inflammatory cytokines, -generation of IL-1β and its mRNA but upregulates A20 (a zinc transcription factor).[32] Taken together, these previous reports thus explain our present observation of suppressed pro-inflammation cytokine (IFN-γ) in most discharged COVID-19 patients after treatment with repurposed drugs containing antioxidant micronutrients (vitamin D, vitamin C and Zn).

Conclusion

Taken together, higher IL-4 and IFN-γ levels in admitted COVID-19 patients compared with discharged COVID-19 patients observed in our study might indicate reduced cytokine storm in discharged patients. However, to prove it broadly, larger panel of cytokine in a larger population of COVID-19 patients is needed to be examined.

Declarations

Funding

The project was self-funded.

Author contributions

Conceptualization, G.O.A., A.A.O., K.A. and M.B.O.; Methodology, G.O.A., A.A.O., K.A. and M.B.O.; Formal Analysis, G.O.A., A.A.O., K.A. and M.B.O.; Data Curation, G.O.A. and A.A.O.; Writing – Original Draft Preparation, G.O.A., A.A.O., K.A. and M.B.O.; Writing – Review & Editing, G.O.A., A.A.O., K.A. and M.B.O.; Project Administration, G.O.A. and A.A.O.

Conflict of interests

The authors declare no competing interests.

Data availability

For ethical reasons, the datasets generated during and/or analysed during the study are confidential. However, they would be available by the corresponding author on genuine request.

Ethics approval

Ethical approval was granted by the Institutional Ethical Committee with approval number UI/EC/20/0283.

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