Natl. Acad. Sci. Lett. (July–August 2022) 45(4):363–369
https://doi.org/10.1007/s40009-022-01123-9

NEWS/VIEWS AND COMMENTS

Th1/Th2/Th17 Cytokine Profile among Different Stages of COVID-19 Infection

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Abstract

Aim To characterize Th1/Th2/Th17 cytokine profile (IL-2, IL-4, IL-6, IL-10, TNF-α, IFN-γ, and IL-17A) among different stages of COVID-19 infection.

Methods This was a cross-sectional study which included six healthy individuals and 68 patients who were admitted with COVID-19 in the Department of Medicine, at All India Institute of Medical Sciences, New Delhi, from July 2020 to September 2020. Patients were categorized into mild, moderate, and severe COVID-19 groups, and serum samples were drawn for the measurement of Th1/Th2/Th17 cytokines (IL-4, IL-6, IL-10, TNF-α, IFN-γ, and IL-17A) which was done by BD™ Cytometric Bead Array.

Results All the cytokines showed dynamic expression in the COVID-19 group, of which only IL-6 was statistically significant. Among the three severity groups of COVID-19, increased severity did not transform into increased cytokine level, with the exception for IL-6, which was statistically significant.

Conclusions In our small sample study, six cytokines expressions were evaluated however most of them were elevated in COVID-19 patients but were not statistically significant except IL-6.

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Keywords Th1/Th2/Th17 · Cytokine profile · COVID-19 · Severity · SARS-CoV-2

Introduction

The World Health Organization (WHO) has declared the novel Coronavirus disease COVID-19 as global pandemic and it has become one of the leading causes of morbidity and mortality worldwide [1]. Every year countries witness sharp rise in the number of new cases and the efforts to overcome the virus are hampered due to the lack of knowledge of several important aspects of SARS-CoV-2 infection [1–3]. Thus, there is an urgent need to dissect the host–pathogen interaction and immune response to the virus, and the contributing immune dysregulation in disease severity. As the preliminary pathobiological studies have revealed that SARS-CoV-2 infection causes tissue destruction thus triggering a local immune response to recruit the macrophages and monocytes to withhold the infection at the site by releasing cytokines and prime the T and B cell against SARS-CoV-2 virus [4, 5].

In most of the cases, an adequate immune response is able to tackle the infection. In some cases unfortunately, immune dysregulation leads to cytokine storm which leads to severe lung injury which is associated with increased COVID-19 associated mortality [6]. Usually after around one week of onset of infection, both the T and B cell against SARS-CoV-2 are detected in the blood. CD4 + T cells are essential in priming both CD8 + T cells and B cells and CD8 + T cells directly attack and kills the virus-infected cells [4, 7]. The cytokine production is also done by CD4 + T cells which further drives the immune cell recruitment [8, 9]. Studies have revealed accumulation of T cells in organs like lungs which suggests these cells are

driven to the site of infection for control of virus and thereby leading to lymphopenia and reduced functional diversity. The severity of COVID-19 is proportionally related to T cell exhaustion and reduced functional diversity of these cells [10].

Due to differences in immunological responses against COVID-19, the infected patients may fall in different clinical spectrum of infection ranging from asymptomatic to severe COVID-19 infection. The cytokine storm associated with COVID-19 infection may vary with the severity of disease. Thus, this study is an attempt to characterize Th1/Th2/Th17 cytokine profile (IL-4, IL-6, IL-10, TNF-α, IFN-γ, and IL-17A) among different severity of COVID-19 infection.

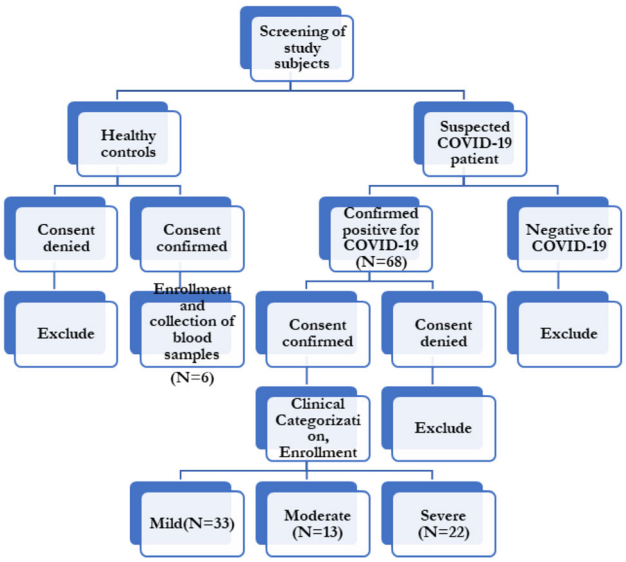
Material and Methods

Subjects

This was a cross-sectional study approved by the Institutional Ethics Committee of All India Institute of Medical Sciences, New Delhi. The study included a total of 68 patients who were admitted with COVID-19 in the Department of Medicine, at All India Institute of Medical Sciences, New Delhi, from July 2020 to September 2020. The study included all patients with age $>$ 18 years, infected with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection confirmed by real-time reverse-transcription-polymerase chain reaction (RT-PCR) assay of nasal, pharyngeal, or lower respiratory tract samples. We extracted medical records and charts of each patient and reviewed all the data. All baseline serum samples were collected immediately after hospital admission and were further divided into mild (n = 33), moderate (n = 13), and severe (n = 22) according to clinical guidance for management of adult COVID-19 patients, Ministry of Health & Family Welfare, Government of India [11]. The serum samples collected from six healthy volunteers served as controls in the study.

Laboratory Examination of Blood Samples

The measurement of Th1/Th2/Th17 cytokines (IL-4, IL-6, IL-10, TNF-α, IFN-γ, and IL-17A) was done by BD™ Cytometric Bead Array (CBA) using Human Th1/Th2/Th17Cytokine Kit according to the manufacturer's protocol.



Statistical Analysis

The data was analyzed by STATA 15.0 software, presented in mean (SD) and frequency percentage. Continuous variables were compared among using one-way ANOVA followed by post hoc comparison which was done using the Bonferroni test and chi-square was used for categorical variable. Correlation among two continuous variables was calculated using Spearman correlation coefficient. p value ($p < 0.05$) was taken as statistically significant.

Results

A total of 74 individuals were recruited for this study, of which six were healthy individuals (controls), 33 suffered from mild infection, 13 from moderate infection, and 22 from severe infection. Moderate and severe infection was predominantly seen in the higher age groups; however, the study was underpowered to detect such differences. Severity of COVID-19 infection had a positive association of comorbidities like diabetes mellitus (DM), hypertension (HTN), chronic kidney disease (CKD), and coronary artery diseases (CAD). However, only DM had a statistically significant (p = 0.03) association with severe COVID-19 disease. The most predominant symptom was fever among all, whereas sore throat which was the next commonly encountered symptom in the mild group, whereas shortness of breath and cough was the next commonly encountered symptom in the mild to moderate COVID-19 group as listed in Table 1.

Table 1 Baseline clinical characteristics of healthy controls and COVID-19 patients

Variables	Healthy	Mild	Moderate	Severe	*p* value
No. of subjects/patients	6	33	13	22	–
Age	35.66 ± 7.6	28.69 ± 11.36	52.16 ± 17.9	51.31 ± 16.95	< 0.001
Underlying comorbidities					
Hypertension	0	1 (3.03%)	1(7.69%)	4(18.18%)	0.133
Diabetes	0	0	3(23.08%)	6(27.27%)	0.03
Coronary artery disease	0	1 (3.03%)	1(7.69%)	1(4.55%)	0.768
Chronic Renal Disease	0	0	2(15.38%)	3(13.64%)	0.033
Chronic Liver Disease	0	0	0	2 (9.09%)	0.136
Malignancy	0	0	0	2 (9.09%)	0.136
Past H/O TB	0	0	0	2 (9.09%)	0.136
Clinical presentation					
Fever	0	27(81.82%)	11 (84.62%)	22 (100%)	0.087
Sore Throat	0	25 (75.76%)	5 (38.46%)	8(36.36%)	0.006
Nausea/ Vomiting	0	4 (12.5%)	0	0	0.127
Generalized Weakness	0	15 (45.45%)	8 (61.54%)	9 (42.86%)	0.589
Shortness of Breath	0	1 (3.03%)	8 (61.54%)	14 (63.64%)	0.000
Headache	0	15 (45.45%)	8 (61.54%)	9 (40.91%)	0.559
Cough	0	19 (57.58%)	10 (76.92%)	16 (72.73%)	0.391
Confusion	0	0	0	2 (9.09%)	0.136
Myalgia	0	15 (46.88%)	8 (61.54%)	9 (40.91%)	0.528
Diarrhea	0	0	2 (15.38%)	0	0.035

Inflammatory Makers in COVID-19 Patients and Controls

We compared the results of IFN-γ, IL-17A, TNF-α, IL-10, IL-6, and IL-4 in serum samples from the control group and COVID-19 patients. Overall, cytokine levels were found to be higher in the COVID-19 group as compared to the control group, however not statistically significant as shown in Fig. 1. The levels of IL-6 were higher in COVID-19 patients when compared to controls ($p = 0.03$). The median value of c-reactive protein (CRP) in controls was 0.55 mg/L while in COVID-19 patients was 2.3 mg/L. However, the difference in both the groups was not statistically significant.

Inflammatory Makers in COVID-19 Patients with Different Severity Levels

In the study, in order to determine whether higher inflammation was associated with severe disease, analysis was done for the cytokine levels of the patients suffering from COVID-19 infection who were sub-grouped based on disease severity. There was no association between IFN-γ, IL-17A, TNF-α, IL-10, IL-6, and IL-4 with respect to the disease severity in COVID-19 patients in Fig. 2.

Correlations Between Serum IL-6 with C-Reactive Protein (CRP) Concentration

There was a positive correlation between IL-6 levels and CRP (Fig. 3) but it was not statistically significant ($r = 0.1$; $p = 0.2$).

Discussion

Ever since the beginning of the COVID-19 pandemic since 2019, attempts have been made to identify the determinants for the severe form of the disease. It was evident early on that the presence of comorbidities such as HTN, DM, CAD, CKD, malignancy, autoimmune disease, and immune-suppression due to transplantation contributed to a more severe form of the disease [12, 13]. Such patients manifested a myriad of clinical features including ARDS and multi-organ failure. The pulmonary vasculature showed evidence of endothelial edema with presence of varying degree of thrombo-emboli in light of all these finding, a suspicion of cytokine storm affecting multiple organ systems arose, similar to the pathological course of hemophagocytic lymphohistiocytosis, which was later on validated by a number of studies [7]. Early investigations

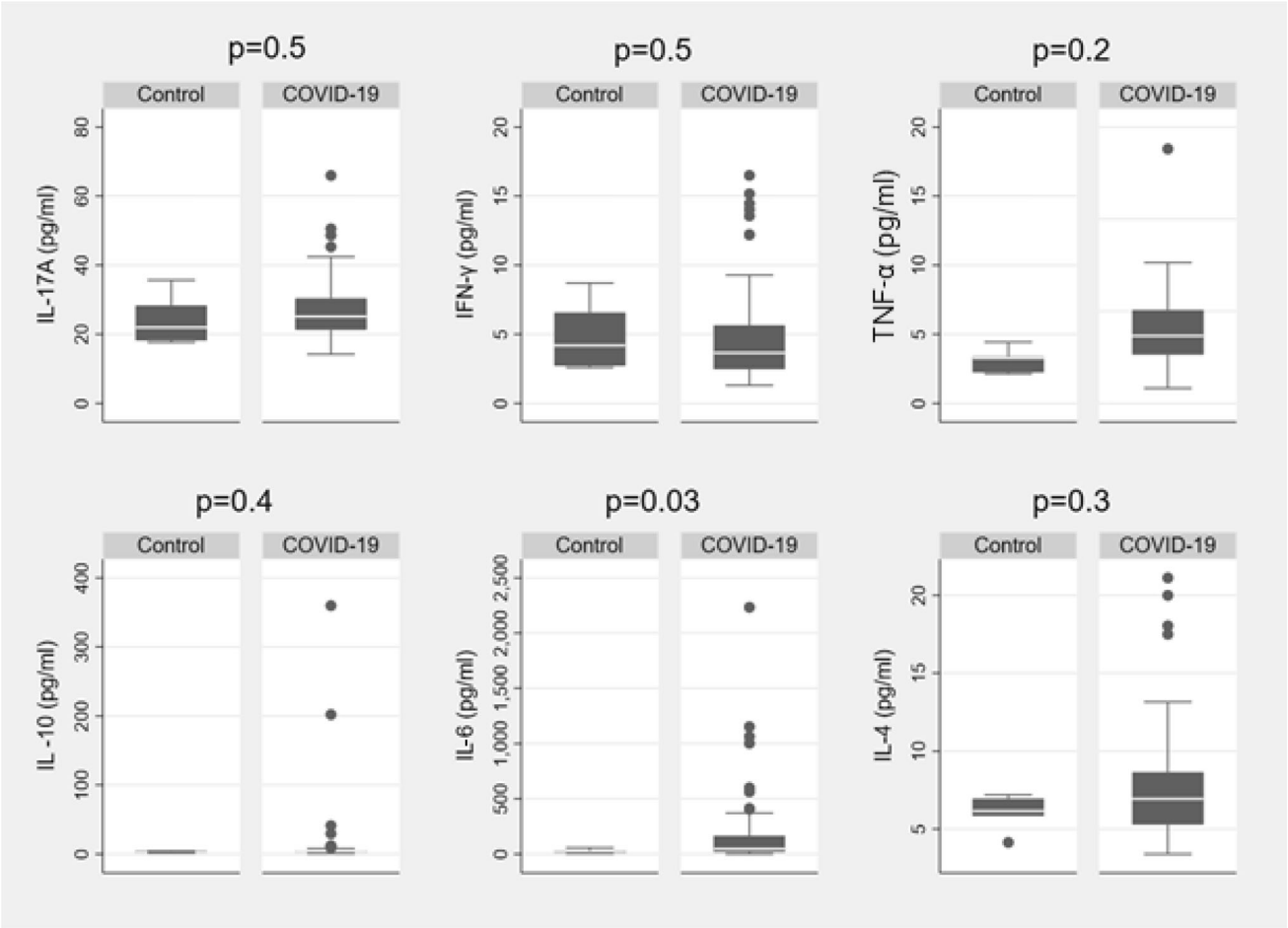


Fig. 1 Cytokine level profiles of healthy control and COVID-19 patients. The serum concentration of TNF-α, IFN-γ, IL-4, IL-6, IL17A, and IL-10 was analyzed

into the cytokine profile showed evidence of a cytokine storm with a fundamental role of T cells in those individuals suffering from the moderate and severe forms of this viral disease. T cells play a fundamental role by resolving viral infections via providing help to B cells for antibody production and orchestrates the response of other immune cells, while the cytotoxic CD8 + T cells perform the function of killing infected cells thereby reducing the viral load. Dysregulation of T cell responses can result in hyper immune activation and commonly studied cytokines found to be abnormally expressed in such individuals were IFN-γ, IL-17A, TNF-α, IL-10, IL-6, and IL-4 along with CRP and ferritin [14]. Different studies detected varying degree of abnormal expression of these molecules and were associated with poor outcome in severe forms of the disease [3, 15, 16]. In our study, six cytokines expressions were evaluated however most of them were elevated in COVID-19 patients but were not statistically significant except IL-6 levels.

An increase in production of IFN-γ indicates a response of Th1 cells due to SARS-CoV-2 infection. Since in our study we have only recorded IFN-γ at the time admission which was higher in patients with mild COVID-19 in comparison to moderate and severe disease. In our small sample study, no difference in the IFN-γ levels between mild, moderate, and severe COVID-19. Gadotti et al. demonstrate rise of IFN-γ response within ten days of illness in patients with COVID-19 may have better outcome but persistent elevated IFN-γ beyond ten days of illness is associated with poor outcome [17]. However, Chinese study by Lui et al. demonstrated in his longitudinal study IFN-γ, together with IL-6 and IL-10, increased in patients with a severe COVID-19 in comparison to those with a mild disease [18]. By contrast, another Chinese study reported that lower levels of IFN-γ in severe disease compared to those with a moderate disease [19]. A strong initial IFN-γ response may lead to decrease in severity of disease and a better outcome in patients with COVID-19 [14].

Studies have demonstrated an increase in Th2 cytokine level (IL-5, IL-6, and IL-9) in COVID-19 patient [14, 20–22]. Guo et al. demonstrated 33 cytokines, which

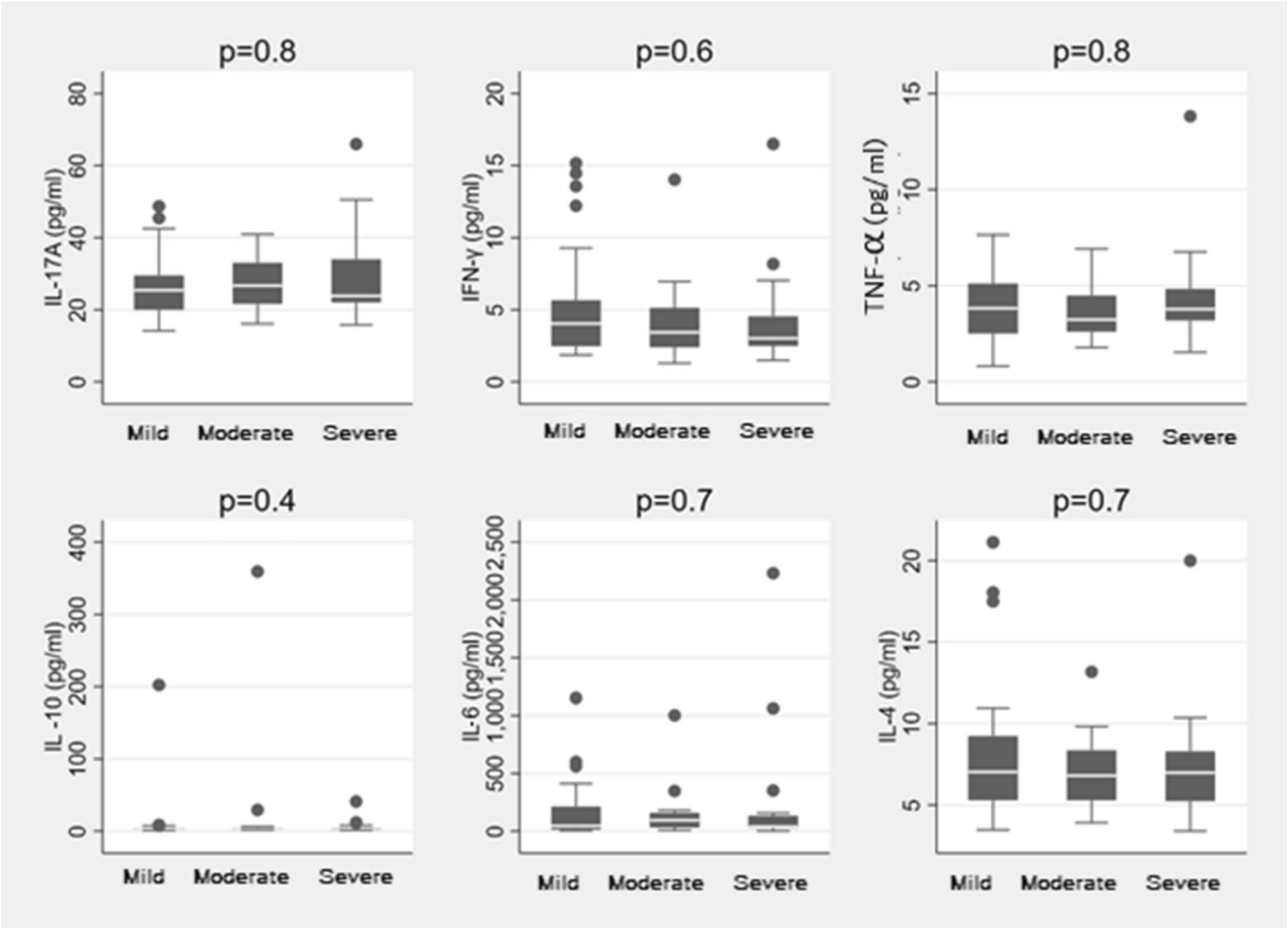


Fig. 2 Cytokine level profiles of mild, moderate, and severe COVID-19 patients. The serum concentration of TNF-α, IFN-γ, IL-4, IL-6, IL17A, and IL-10 was analyzed

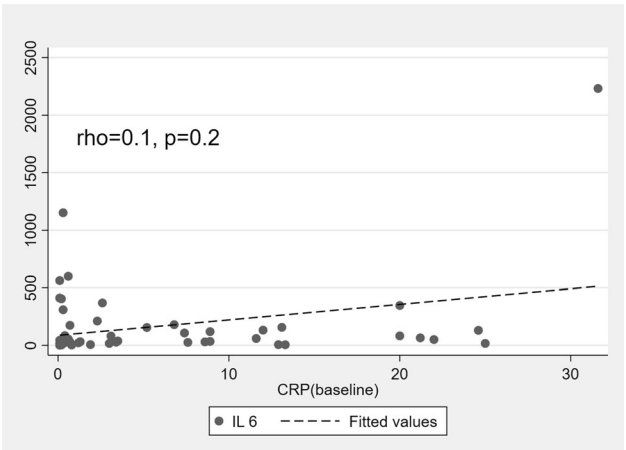


Fig. 3 Scatter diagram depicts the relationship between IL-6 and CRP. The relationship between CRP and IL-6. Spearman rank correlation analysis was performed to evaluate the correlation of serum IL-6 with CRP in the patients with COVID-19

were both anti-inflammatory cytokines (such as IL-10 and IL-13) and pro-inflammatory cytokines (such as IL-1β, IL-6, IP-10, G-CSF, IL-8, IL-17, and IFN-γ) were significantly increased in COVID-19 patients [21]. In our study, these cytokines were found to have increased expression in the COVID-19 patients, however, of which only IL-6 was statistically significant ($p = 0.03$) when compared with healthy controls. Huan et al. described higher levels of TNF-α, IFN- γ, IL-2, IL-4, IL-6, and IL-10, CRP in the moderate and severe COVID-19 infection group, however only IL-6, IL-10 showed increased expression along with disease severity [23]. Parimoo et al. described the higher IL-6 levels were significantly associated with an adverse final outcome ($p = 0.007$) [22]. Studies worldwide have published the association of higher IL-6 level with severe disease and adverse outcomes [15, 16, 22–25]. Thus, blockade of the IL-6 signaling pathway would help in decreasing the progression of the COVID-19 infection to more severe forms, which was amply exemplified by the usage of IL-6/ IL-6R blocking agents in improving the clinical outcomes in the form of improved ventilatory parameters, improved inflammatory markers, and decreased hospital stay [26].

IL-17 is the key cytokine which is a potent neutrophil chemoattractant and neutrophil response regulator [10]. In our study levels of IL-17 increased in moderate and severe cases compared to the mild group and healthy control group. Guo et al. demonstrated pro-inflammatory cytokines (such as IL-1β, IL-6, IP-10, G-CSF, IL-8, IL-17, and IFN-γ) were significantly increased in COVID-19 patients [21].

Limitation

There were several limitations in our study. The sample size was inadequate to detect the significant differences in the cytokine levels among the different groups. Secondly, serial cytokine levels could not be done for individual patients to determine the cytokine kinetics in relation to the disease progression. And lastly, the effect of comorbidities on the cytokine profile could not be determined due to its skewed distribution among the different groups.

Conclusions

In our small sample study, six cytokines expressions were evaluated however most of them were elevated in COVID-19 patients but were not statistically significant except IL-6. This study suggests increase of pro-inflammatory cytokines in COVID-19 patients and may be helpful in development of predictive models to predict severe disease at early stages SARS-CoV-2 infection. Thus, definitive approaches for treatments can be initiated.

Acknowledgements We would like to express our thanks to residents and nursing staff of Department of Medicine, AIIMS, New Delhi, for facilitating the study.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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