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Original Article

Imbalance of T Helper Cell Subset Specific Transcription Factors and Associated Cytokines in Patients with Severe COVID-19

Priyal Gupta[1#], Kajal Patel[1#], Garima Garg[1], Bijina J. Mathew[1], Dipesh Kale[1], Jitendra Singh[2], Sagar Khadanga[3], Shashank Purwar[1], Debasis Biswas[1], Anirudh K. Singh[1] and Ashish Kumar Vyas[1,4*]

[1]Department of Microbiology, All India Institute of Medical Sciences, Bhopal, India; [2]Department of Translational Medicine, All India Institute of Medical Sciences, Bhopal, India; [3]Department of Medicine, All India Institute of Medical Sciences, Bhopal, India; [4]Department of Immunology, JC Martin Center for Liver Research and Innovations, Liver Foundation West Bengal, India

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Abstract

Background and aims: Coronavirus disease (COVID-19) exhibits a range of clinical symptoms, including viral pneumonia, which can progress to acute respiratory distress syndrome, substantial alveolar destruction, and even multi-organ failure in severe cases. Disease pathology is greatly influenced by the host immune response. Several studies reported the perturbation of T-cell responses in COVID-19 patients. Activation and differentiation of CD4+ T cells into various subsets depend on the expression of lineage-specific transcription factors and overall cytokine milieu. Hence, a thorough evaluation of T helper cell lineage-specific transcription factors and pro-inflammatory cytokines can provide crucial insight into COVID-19 pathogenesis and may aid in developing strategies to prevent disease severity. Here, we performed a cross-sectional study to delineate the dysfunctional T helper cell subset immune response associated with COVID-19 disease severity.

Methods: We assessed T helper cell responses in SARS-CoV-2 infected individuals who presented with either asymptomatic, mild, or severe disease. mRNA profiling of lineage specific transcription factors and associated cytokines was done using real-time qPCR. Cytokine profiling was done using ELISA.

Results: mRNA levels of FOXP-3 were significantly decreased in patients with severe COVID-19. No significant difference was observed for T-bet and GATA-3 among all of the groups. Bcl-6, the transcription factor for Tfh cell subsets, showed an increased trend in its association with disease progression. Furthermore, mRNA levels of IL-21 were significantly increased with disease severity. We also observed a significant increase in the concentration of pro-inflammatory cytokines IL-6 and IL-1β in patients with severe COVID-19.

Conclusions: These findings provide new insight into COVID-19 disease pathology and may aid in developing effective strategies to manage/control disease severity.

Keywords: Covid-19; Immune Responses; T helper Cell; Cytokine storm.

Abbreviations: ARDS, Acute respiratory distress syndrome; FOX-P3, Forkhead box P3; GC, germinal center; IFN, interferon; IL, interleukin; MCP-1, monocyte chemoattractant protein-1; Tfh, T follicular helper; Th, T helper; Treg, T regulatory; TGF-β, tumor growth factor-β; SARS-CoV-2, Severe Acute Respiratory Syndrome Coronavirus 2.

*Correspondence to: Ashish Kumar Vyas, Department of Microbiology, All India Institute of Medical Sciences, Saket Nagar, Bhopal 462024, India. ORCID: https://orcid.org/0000-0001-6333-0195. Tel: 09540946986, E-mail: a88_ashish@yahoo.co.in
#These authors contributed equally to this work.

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Introduction

Coronavirus disease (COVID-19) is caused by severe acute respiratory syndrome Coronavirus 2 (SARS-CoV-2). The first case of COVID-19 was reported in China in December 2019 and was then officially declared a pandemic by the World Health Organization in March 2020. The epidemic has affected almost every aspect of society and remains an on-going health crisis. SARS-CoV-2 primarily affects the human respiratory tract exhibiting symptoms such as cough, breathlessness, chest pain, fever, and fatigue.

SARS-CoV-2 infection shows various clinical manifestations, including viral pneumonia, that can ultimately progress to acute

respiratory distress syndrome, extensive alveolar damage,[1] and even multi-organ failure in severe cases.[2] It has been suggested that the host immune response plays a significant role in disease pathogenesis, thereby impacting clinical outcomes.[3] Several studies reported the perturbation of T cell responses in COVID-19 patients. Lymphopenia has been considered a hallmark of COVID-19 severity,[4,5] as the total count of T cells, CD4+ T cells, and CD8+ T cells were found to be significantly low in intensive care unit (ICU) COVID-19 cases compared to non-ICU cases.[6] One study reported the infiltration of lymphocytes in the lung tissue of COVID-19 patients.[7] In addition, T cells were found to be functionally impaired in patients with severe COVID-19 and were associated with the overexpression of exhaustion markers such as Tim 3 and PD-1.[8]

The ability of activated CD4+ T cells to differentiate into various functional subsets depends on the differentiation and secretory profiles of various transcriptional factors and cytokines. CD4+ T cells can differentiate into T helper (Th) 1, Th2, Th17, T follicular helper (Tfh), or T regulatory (Treg) cells. A cytotoxic T cell response is mediated through the production of interferon-γ (IFN-γ) via Th1 subsets. In contrast to Th1 subsets, Th2 subsets produce interleukin (IL)-4 and IL-6. The master regulators of these subsets are T-bet and GATA-3 for Th1 and Th2 cell subsets, respectively. Activation of Th1 and Th2 responses can lead to ahyperinflammatory environment. Therefore, it is important to regulate and maintain the balance of immune responses. Tregs, which are a specialized subset of Th cells, play a key role in regulating cell balance by creating an immunosuppressive environment. CD4+ T cells differentiate into Tregs via the transcription factor Forkhead box P3 (FOX-P3). Tfh cells also play an important role in the germinal center (GC) response, leading to the activation of B cells and the subsequent production of neutralizing antibodies. Bcl-6 plays a major role in differentiation of Tfh cells. Activation of this subset leads to the production of several pro-inflammatory cytokines. In some patients, over-production of Tfh creates a cytokine storm which leads to multi-organ failure associated with high mortality in patients with severe COVID-19.

Thus, an evaluation of T cell mediated immune responses can provide crucial insight into COVID-19 pathogenesis and may aid in developing strategies to prevent disease severity. Here, we performed a cross-sectional study to delineate Th cell subset-mediated immune responses associated with COVID-19 disease severity.

Materials and methods

Ethics statement

This study was approved by the Institutional Human Ethics Committee of All India Institute of Medical Sciences, Bhopal (Approval No. IHEC-LOP/2020/EF0184 dated May 5th, 2020). Samples were collected from the study participants after obtaining written informed consent during the second wave of COVID-19.

Study subjects

A cross-sectional study was performed on 45 patients with COVID-19 disease. All COVID-19 patients were confirmed positive for SARS-CoV-2 using RT-PCR of throat/nasal swab samples and were hospitalized at All India Institute of Medical Sciences, Bhopal. Concurrently, 15 age-matched individuals with no travel or prior contact history and who did not present with any symptoms were recruited as the healthy control group in this study. Grouping of the participants was done as described here.

Control group: participants (n = 10) were confirmed negative for SARS-CoV-2 using RT-PCR and presented with no signs or symptoms of COVID-19 disease.

Asymptomatic group: participants (n = 16) were confirmed positive for SARS-CoV-2 using RT-PCR, but did no present with any signs or symptoms of COVID-19 disease.

Mild group: participants (n = 13) were confirmed positive for SARS-CoV-2 using RT-PCR and presented with mild symptoms of COVID-19 disease, including fever and cough, but did not require oxygen support or mechanical ventilation.

Severe group: participants (n = 17) were confirmed positive for SARS-CoV-2 using RT-PCR and presented with severe symptoms of COVID-19 disease, including shortness of breath and chest pain, and required oxygen or mechanical ventilation; these participants were admitted to the ICU.

Sample collection and RNA extraction

Venous blood (4–5 mL) was collected in EDTA tubes. RNA was extracted from the blood using the QIamp Blood RNA isolation kit according to the manufacturer's protocol. RNA was quantified using Nanodrop. Plasma was separated within an hour of sample collection and stored immediately at −80°C until use.

cDNA synthesis and quantitative RT-PCR

A total of 500 ng of RNA was used to prepare cDNA. The cDNA library was made using random hexamers in the ABI cDNA synthesis kit (Applied Biosystems) according to the manufacturer's protocol. Quantitative RT-PCR was performed using Power up SYBR Green (ABI Master mix) with gene specific primers (Table 1) with the BioRad CFX 96 real-time PCR (BioRad). The cycling conditions used were as follows: denaturation at 95°C for 20 s, annealing temperature according to the primers used for 20 s, and extension at 60°C for 20 s. Melt curve was incorporated to assess and ensure the specificity of PCR products. GAPDH was used to normalize gene expression. Reactions were set in duplicate, and the average of the two Ct values was used for further analyses.

ELISA

Circulating concentrations of IL-6, IL-1β, and monocyte chemoattractant protein-1 (MCP-1) of each sample were measured in duplicate using CUSABIO ELISA kits according to the manufacturer's protocol.

Statistical analysis

All statistical tests were performed using GraphPad Prism for Windows version 8.0.1. Participant demographic data are presented as either the median with range or mean with standard deviation. Multiple comparisons between groups were performed using the Kruskal-Wallis test with Dunn's correction. All statistical comparisons were two-tailed and a *P*-value of <0.05 was considered significant.

Results

Clinical characteristics of study subjects

The demographic and clinical characteristics of COVID-19 patients are reported in Table 2. There were no significant differences in age, blood pressure, pulse rate, or hemoglobin level among the groups. However, the respiratory rate increased with disease severity. In accordance with the published literature, disease severity

Table 1. Primer sequences for Th subset specific transcription factors and pro-inflammatory cytokines

S. No.	Primer name	Primer sequence 5′-3′	Annealing temperature	Product length
1	T BET (F)	ACAACCACCTGTTGTGGTCC	58°C	104 bp
	T BET (R)	CCCGGCCACAGTAAATGACA		
2	IFN γ (F)	GAGTGTGGAGACCATCAAGGAAG	66°C	124 bp
	IFN γ (R)	TGCTTTGCGTTGGACATTCAAGTC		
3	GATA 3 (F)	TCATTAAGCCCAAGCGAAGG	58°C	107 bp
	GATA 3 (R)	GTCCCCATTGGCATTCCTC		
4	IL-6 (F)	TGAGGAGACTTGCCTGGTGA	60°C	109 bp
	IL-6 (R)	CACAGCTCTGGCTTGTTCCT		
5	TGF-β (F)	CAGCAACAATTCCTGGCGATA	55°C	136 bp
	TGF-β (R)	AAGGCGAAAGCCCTCAATTT		
6	FOX-P3 (F)	CACCTGGCTGGGAAAATGG	56°C	63 bp
	FOX-P3 (R)	GGAGCCCTTGTCGGATGA		
7	BCL-6 (F)	CTGCAGATGGAGCATGTTGT	58°C	92 bp
	BCL-6 (R)	TCTTCACGAGGAGGCTTGAT		
8	IL-21 (F)	TATGTGAATGACTTGGTCCCTGA	62°C	106 bp
	IL-21 (R)	AGCTGACCACTCACAGTTTGT		
9	GAPDH (F)	GTCTCCTCTGACTTCAACAGCG	66°C	131 bp
	GAPDH (R)	ACCACCCTGTTGCTGTAGCCAA		

IFN, interferon; IL, interleukin; TGF-β, tumor growth factor-β.

was found to be associated with leukocytosis and lymphopenia in patients with severe COVID-19.[4] Aspartate aminotransferase and alanine transaminase levels were also found to be associated with disease severity, consistent with the literature.[9] Serum creatinine levels were in the normal range for all groups. In agreement with the literature, inflammatory markers, such as C-reactive protein and lactate dehydrogenase were increased in patients with severe COVID-19.[10]

Expression of Th1 and Th2 specific transcription factors and cytokines are perturbed in patients with severe COVID-19

Differentiation of Th1 and Th2 subsets is regulated by T-bet and GATA-3 transcription factors. We measured the expression level of these factors to determine the differentiation potential of Th1 and Th2 subsets, respectively. We found that T-bet mRNA levels were decreased in all groups compared to the Control group (Control 1.40 ± 0.34 *vs* Asymptomatic 0.78 ± 0.25, *vs* Mild 0.51 ± 0.15, *vs* Severe 0.69 ± 0.37). GATA 3 mRNA levels were also decreased in all groups compared to the Control group (Control 1.22 ± 0.25 *vs* Asymptomatic 0.65 ± 0.18, *vs* Mild 0.89 ± 0.32 *vs* Severe 0.53 ± 0.2). However, these changes were not statistically significant (Fig. 1a, b).

We further assessed the expression of effector cytokines IFN-γ and IL-6. We found that levels of IFN-γ, but not IL-6, were decreased in all groups compared to the Control group (Control 1.48 ± 0.63 *vs* Asymptomatic 1.09 ± 0.61, *vs* Mild 0.26 ± 0.07 *vs* Severe 0.64 ± 0.27). IL-6 mRNA levels were associated with disease severity; there was a significant increase in IL-6 levels in patients with severe COVID-19 compared to the Control groups (Control 2.24 ± 0.94 *vs* Asymptomatic 5.12 ± 1.724, *vs* Mild 6.9 ± 1.99, *vs* Severe 8.94 ± 1.67; $P = 0.018$) (Fig. 1c, d).

Treg-specific transcription factor and effector cytokine are altered in patients with severe COVID-19

The differentiation potential towards Tregs was analyzed by measuring the expression of its transcription factor, FOXP3. FOXP3 was significantly downregulated in the Severe group compared to the other groups (Control 1.1 ± 0.2 *vs* Asymptomatic 1.05 ± 0.21, *vs* Mild 1.2 ± 0.44, *vs* Severe 0.33 ± 0.07; $P = 0.037$) (Fig. 2a). The expression of its effector regulatory cytokine tumor growth factor-β (TGF-β) was highly variable among the groups (Control 1.21 ± 0.24 *vs* Asymptomatic 1.38 ± 0.22, *vs* Mild 2.5 ± 0.9, *vs* Severe 1.92 ± 0.97) (Fig. 2b).

Tfh-specific transcription factor and its related cytokine are perturbed in patients with severe COVID-19

We next assessed the expression of Bcl-6, which is responsible for Tfh cell differentiation. Bcl-6 was increased in all three groups compared to the Control group (Control 1.18 ± 0.20 *vs* Asymptomatic 2.39 ± 0.5, *vs* Mild 3.7 ± 1.4, *vs* Severe 1.7 ± 1.0) (Fig. 3a). Subsequently, expression of its effector cytokine, IL-21, was also found to be increased with disease progression. IL-21 was significantly upregulated in the Severe group compared to the Control group (Control 1.29 ± 0.27 *vs* Asymptomatic 2.00 ± 0.4, *vs* Mild 3.03 ± 0.76, *vs* Severe 4.22 ± 0.73; $P = 0.02$) (Fig. 3b).

Cytokine profiling suggests a pro-inflammatory environment in patients with symptomatic COVID-19

Cytokine storm is a marked feature of disease severity. Therefore, we also measured concentrations of circulating pro-inflammatory cytokines using ELISA. In accordance with the literature, we ob-

Table 2. Baseline demographic and biochemical data among the groups

Parameter	Asymptomatic (n = 16)	Mild (n = 13)	Severe (n = 17)
Age (Year) *	52.31 (24–84)	48.75 (38–75)	52.7 (34–72)
Male (%)	68.75%	76.9 %	70.5%
PR (Beats/minute)	89.3 ± 17.8	99.6 ± 17.9	90.0 ± 27.7
RR (Breaths/ minute)	25.2 ± 4.5	25 ± 7.02	28.0 ± 6.6
Systolic blood pressure (SBP) (mmHg)	122.5 ± 14.4	130.5 ± 19.7	129.0 ± 16.2
Diastolic blood pressure (SBP) (mmHg)	74.5 ± 11.7	80 ± 12.5	83.0 ± 12.2
Hemoglobin (g/dL)	13.3 ± 1.64	13.9 ± 1.5	13.6 ± 1.1
MCV (FL)	84 ± 2.8	80.7 ± 33.6	93.0 ± 6.3
TLC (x10^3/µl)	6,958.3 ± 1,680.5	7,096.6 ± 7,515.2	6,334 ± 2,526.6
NEUTROPHIL (%)	62 ± 10	72.3 ± 18.5	73.7 ± 9.9
LYMPHOCYTES	28 ± 8.7	19.5 ± 15.85	17.0 ± 7.3
NLR	1.62	1.89	3.2
UREA (mg/dL)	31.7 ± 8.7	40.4 ± 17.06	30.0 ± 15
CREATININE (mg/dL)	1.2 ± 0.56	1.05 ± 0.23	0.9 ± 0.1
SODIUM (mmol/L)	132.7 ± 2.9	137.5 ± 2.1	128.3 ± 6.4
POTASIUM (mmol/L)	3.53 ± 0.23	3.45 ± 0.2	3.8 ± 0.7
AST (SGOT) (U/L)	99.5 ± 127	69.9 ± 44.2	69.0 ± 36.4
ALT (SGPT) (U/L)	140.5 ± 138.5	84.6 ± 65.9	87.9 ± 113.9
BILIRUBIN TOTAL(mg/dL)	0.47 ± 0.10	0.78 ± 0.22	0.7 ± 0.2
CON BILIRUBIN (mg/dL)	0.11 ± 0.03	0.25 ± 0.19	0.2 ± 0.1
ALBUMIN (g/dL)	3.94 ± 0.26	3.76 ± 0.54	3.8 ± 0.3
GLOBULIN (g/dL)	2.9 ± 0.27	3.13 ± 0.49	3.2 ± 0.5
A/G	1.34	1.22	1.2
CRP (mg/L)	6.1 ± 4.38	33.2 ± 19.10	46.9 ± 55.5

ALT, alanine transaminase; AST, aspartate aminotransferase; CRP, C-reactive protein; MCV, mean corpuscular volume; NLR, neutrophils to lymphocyte ratio; PR, pulse pate; RR, respiratory rate; TLC, total leukocyte count.

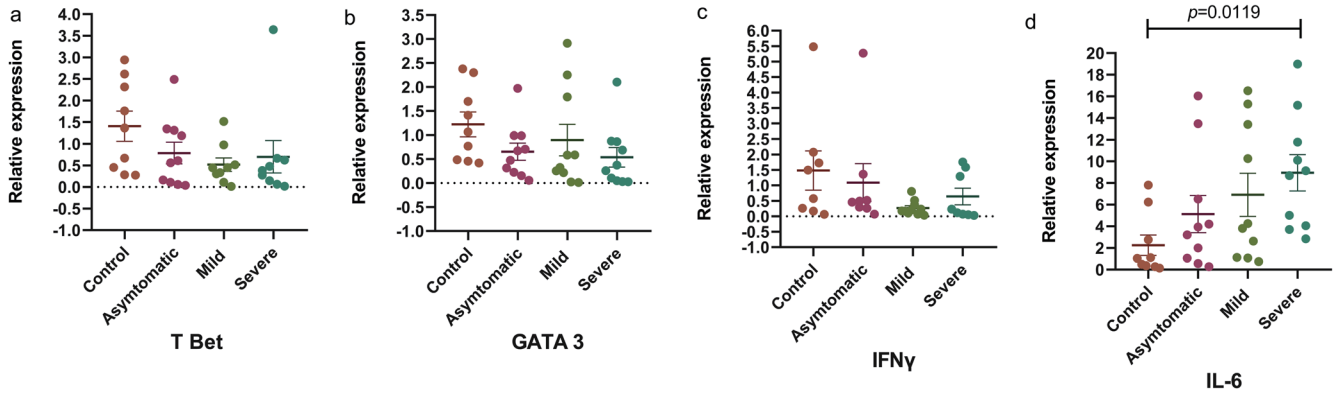

Fig. 1. Relative expression of Th1 and Th2 specific transcription factors and effector cytokines among different COVID-19 groups. mRNA levels of (a) T-bet [Control (n = 9), Asymptomatic (n = 10), Mild (n = 9), and Severe (n = 9)]; (b) GATA-3 [Control (n = 9), Asymptomatic (n = 10), Mild (n = 10), and Severe (n = 10)]; (c) IFN-γ [Control (n = 8), Asymptomatic (n = 8), Mild (n = 8), and Severe (n = 8)]; (d) IL-6 [Control (n = 9), Asymptomatic (n = 10), Mild (n = 10), and Severe (n = 10)]. The data are presented as the mean ± SD. Statistical significance was calculated using the Kruskal-Wallis test. IFN-γ, interferon-γ; IL-6, interleukin 6.

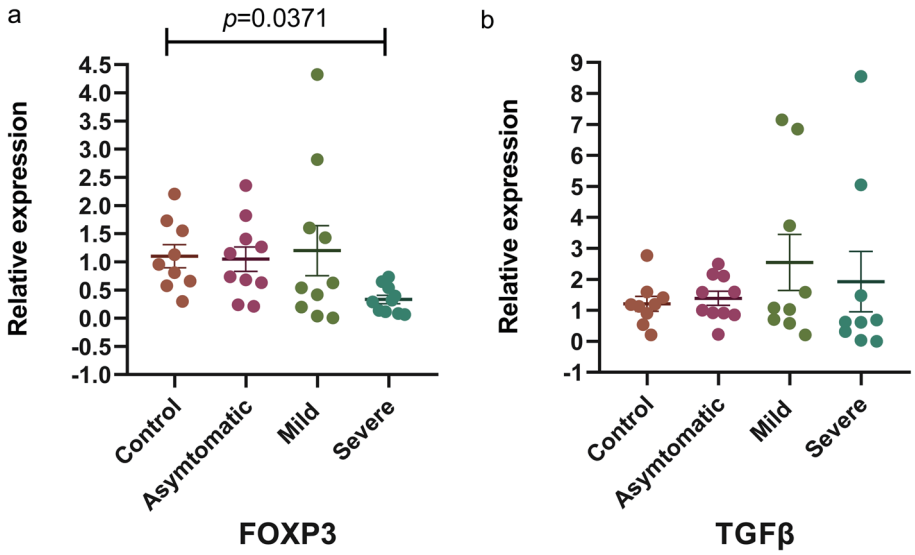

Fig. 2. Relative expression of Treg-specific transcription factor and effector cytokine among different COVID-19 groups. mRNA levels of (a) FOXP3 [Control (n = 9), Asymptomatic (n = 10), Mild (n = 10), and Severe (n = 10)]; (b) TGF-β [Control (n = 9), Asymptomatic (n = 10), Mild (n = 9), and Severe (n = 9)]. The data are presented as the mean ± SD. Statistical significance was calculated using the Kruskal-Wallis test. TGF-β, tumor growth factor-β.

served a significant increase in the concentration of IL-6 with disease severity (Control 6.19 ± 2.28 *vs* Asymptomatic 9.8 ± 3.8, *vs* Mild 45.09 ± 12.00, *vs* Severe 87.72 ± 13.40; $P = 0.0021$) (Fig. 4a). IL-1β was also found to be significantly increased in the Severe group compared to the Asymptomatic and Control groups (Control 18.21 ± 7.18 *vs* Asymptomatic 188.3 ± 21.96, *vs* Mild 243.4 ± 49.56, *vs* Severe 580.9 ± 84.75; $P < 0.0001$) (Fig. 4b). IL-21 was also increased, consistent with our previous findings.[11] MCP-1 was significantly increased in the Severe group compared to the Control group (Control 95.50 ± 14.68 *vs* Asymptomatic 199.9 ± 40.9, *vs* Mild 193.5 ± 46.21, *vs* Severe 304.4 ± 69.92; $P = 0.034$) (Fig. 4c).

Discussion

SARS-CoV-2 infection is responsible for the enhanced inflammatory response leading to cytokine storm and deteriorated health conditions. Several studies revealed dysfunction of different T cell subsets during infection and their role in the pathogenesis and resolution of COVID-19. However, studies probing the associations of T cell subsets with COVID-19 disease severity are limited. In this study, we assessed the Th cell response in individuals infected with SARS-CoV-2 who displayed different disease symptoms, ranging from asymptomatic to mild and severe. The mRNA profiling of lineage-specific transcription factors T-bet, GATA-3, and FOXP-

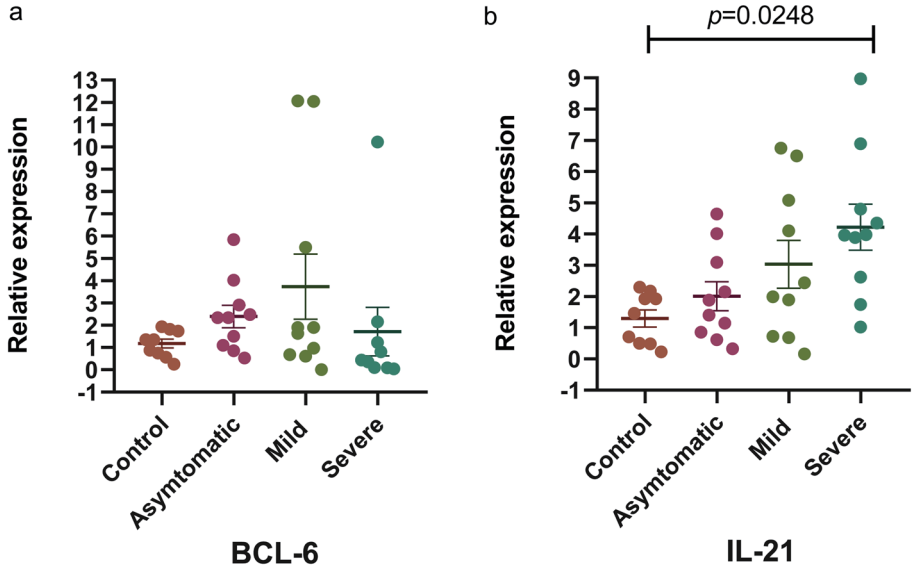

Fig. 3. Relative expression of Tfh-specific transcription factor and effector cytokine among different COVID-19 groups. mRNA expression of (a) Bcl-6 [Control (n = 9), Asymptomatic (n = 10), Mild (n = 10), and Severe (n = 9)]; (b) IL-21 [Control (n = 9), Asymptomatic (n = 10), Mild (n = 10), and Severe (n = 10)]. The data are presented as the mean ± SD. Statistical significance was calculated using the Kruskal- Wallis test. IL-21, interleukin 21.

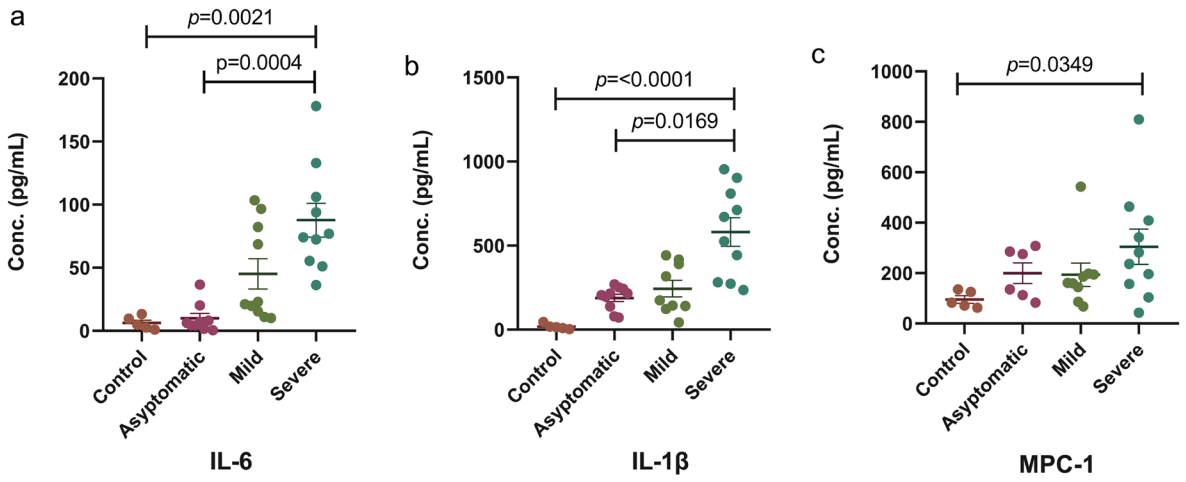

Fig. 4. Concentration of pro-inflammatory cytokines among different COVID-19 groups. Concentration of (a) IL-6 [Control (n = 5), Asymptomatic (n = 8), Mild (n = 10), and Severe (n = 10)]; (b) IL-1β [Control (n = 5), Asymptomatic (n = 8), Mild (n = 9), and Severe (n = 10)]; (c) MCP-1 [Control (n = 5), Asymptomatic (n = 6), Mild (n = 9), and Severe (n = 10)]. The data are presented as the mean ± SD. Statistical significance was calculated using the Kruskal-Wallis test. IL, interleukin; MCP-1, monocyte chemoattractant protein-1.

3, which are responsible for the differentiation of Th1, Th2, and Treg cells, respectively, indicated that expression of these factors decreased with increased disease severity. However, the expression of Bcl-6, which is a transcription factor of the Tfh cell subset, was found to be increased. The mRNA levels of the cytokines associated with these respective T cell subsets (IFN-γ and IL-21) displayed a similar trend. However, mRNA levels of IL-6 were significantly increased. We further observed a significant increase in the concentration of pro-inflammatory cytokines IL-6, IL- 1β and MCP-1 in the circulation of patients with severe COVID-19. We did not find Th1 and Th2 responses to be associated with disease severity, consistent with a previous study.[12] One study showed a poor Th1 response in COVID-19 and a forced Th2 response, while another study reported an imbalance in the Th1 and Th2 response that was associated as an increased risk for COVID-19-linked mortality.[11,12] The observed increase in IL-6 mRNA levels could be due to activation of multiple cell subsets such as mast cells, macrophages, dendritic cells, and T and B cells in response to various infections.[13] However, the increased expression of IL-6 clearly explains failure of the Th1 response and decreased mRNA levels of T-bet and IFN-γ in COVID-19 patients, as IL-6 inhibits Th1 differentiation via interfering with IFN-γ signaling.[14]

Similarly, with respect to Tregs, a single-cell analysis suggested a marked decrease in FOXP-3 expression in patients with severe COVID-19.[15] Another study also reported reduced mRNA levels of FOXP3 among COVID-19 patients admitted to the ICU, which is in concordance with our results.[16] One potential reason for this marked reduction in FOXP-3 expression and Treg population in patients with severe COVID-19 is the overexpression of IL-6, as IL-6 inhibits TGF-β- induced Treg differentiation.[17] A decrease in the immunosuppressive cytokine TGF-β was also previously reported,[18] which might be another reason for the lack of Treg differentiation. However, expression of TGF-β in SARS-CoV-2 infection is very discrepant and has been reported to be increased in the circulation of patients with severe COVID-19, thus limiting the antiviral activity of natural killer cells as indicated in another study.[19,20] These findings indicate a failure of Treg subsets to regulate the inflammatory environment, which could be a potential contributor to COVID-19 severity.

Single-cell analysis has also demonstrated an increased frequency of circulating Tfh cells in patients with COVID-19. However, another study reported defective Tfh cell differentiation with a reduced GC response in patients with severe COVID-19. A reduction in the number of Bcl-6+ GC Tfh cells and an impaired humoral response in patients with severe COVID-19 have also been reported.[21] In our previous study we reported a higher concentration of circulating IL-21 levels among severe cases,[11] consistent with our IL-21 mRNA levels reported here. Several studies suggested that high serum levels of IL-21, but not IL-6, are a predictive indicator for poor prognosis in COVID-19 patients.[22] The high serum concentration of IL-21 could also be due to other contributing cell populations, such as central memory T cells and Th17 cells.[23,24] Similar to our findings, the significance of circulating IL-1β in severity has been established by other studies, targeting it as a therapeutic agent.[25]

The use of several immunomodulating agents as a therapy specifically targeting IL-6 was seen during the peak of the pandemic.[26] However, to date, no conclusive treatment modalities have been devised for COVID-19. In addition to IL-6, several other pro-inflammatory cytokines can contribute to the cytokine storm seen in COVID-19. Our study indicates IL-21 as a potential therapeutic target for COVID-19 disease, as targeting IL-21 might disrupt the associated immunopathology in COVID-19. Pneumonia virus of mice causes respiratory infection leading to an enhanced inflammatory response and shares the same characteristics as severe human respiratory syncytial virus infection. In both infections, IL-21 producing CD4+ T cells accumulate in the lungs. Blockage of this IL-21 accumulation and subsequent signaling with an IL 21R–Fc fusion protein was shown to increase survival of mice.[27] Consequently, several clinical trials have used IL-21 therapy and its blockades for the treatment of certain cancers and other immune-associated disorders.[28–30] Apart from IL-21, IL-1β was also found to be a potential therapeutic target for COVID-19. In one clinical trial, canakinumab, a fully human IgG monoclonal antibody, was used as an IL-1β blocker to treat patients with severe COVID-19. The results indicated a declined inflammatory response and improved oxygenation.[25] Other than targeting cytokines, immune checkpoint inhibition therapy can also be used as the treatment modality for COVID-19 patients with lymphopenia. The upregu-

lated expression of immune checkpoint inhibitors has been seen in COVID-19 and was found to be associated with lymphopenia and T-cell exhaustion.[31] We also found decreased expression of T-bet and GATA-3 indicating potential T-cell exhaustion.

There are some limitation of our study that should be noted. First, our study included a small cohort. Further, due to a lack of sample availability, we were unable to determine the frequency of Th subsets and therefore restricted our study to expression profiling of respective transcription factors and cytokines. While these findings are relevant to understanding disease pathology and may aid in developing effective strategies to manage/control COVID-19 severity, our findings need to be validated with a larger cohort before any efforts are made in this direction.

Conclusion

Using samples from healthy and SARS-CoV-2 infected individuals with varying degrees of disease manifestation, we conclude that patients with severe COVID-19 have impaired T cell responses, as indicated by the altered expression of transcriptional factors responsible for differentiating Th cells. A decrease in Tregs via decreased expression of FOXP-3 in patients with severe COVID-19 may provide an explanation for the excessive inflammation observed in these patients. Our study provides new insight that could be used in the development of targeted therapies for COVID-19.

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Conflict of interest

No conflict of interest is associated among the authors with this publication.

Author contributions

Conceptualization, project administration and funding acquisition (AKV); visualization and original draft preparation (PG and KP); methodology, data curation, and validation (PG, KP, GG, BJM and DK); formal analysis (PG, KP, AKS and AKV); resources (JS, SK, SP and DB); review and edit revised manuscript (AKS and AKV); supervision and investigation (AKV and AKS).

Data sharing statement

The datasets generated during the current study are available from the corresponding author a88_ashish@yahoo.co.in on reasonable request.

Ethical statement

This study was approved by Institutional Human Ethics Committee of All India Institute of Medical Sciences, Bhopal (Approval No. IHEC-LOP/2020/EF0184 dated May 5th, 2020). Samples were collected from the study participants after obtaining written informed consent during the second wave of COVID-19.

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