

The Monocyte Chemotactic Protein 1 as a Potential Indicator for SARS-CoV-2 Infected Mild COVID-19 Patients

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Abstract

Background: The level of chemokine was markedly elevated in severe COVID-19 patients. But the role of chemokine in mild COVID-19 patients has not yet established. However, most of the COVID-19 patients in Shiyuan City, China had mild cases.

Methods: In this study, the level of chemokine in the serum for COVID-19 patients in Shiyuan City was detected by ELISA. The expression of receptor of chemokine and other signal molecular was detected by real-time PCR.

Results: We first demonstrated that COVID-19 patients are characterized by higher levels of chemokine. In particular, monocyte chemoattractant protein 1 (MCP-1) has shown higher expression in patients with mild cases of COVID-19. The receptor of MCP-1, CCR2 was then found higher expression in the same mild COVID-19 patients. Finally, the higher expression of MCP-1 in mild COVID-19 patients is correlated with the inhibition of IFN signaling.

Conclusion: These findings add to our understanding of the immune-pathologic mechanisms of SARS-CoV-2 infection, and provide potential therapeutic targets and strategies. MCP-1 may be an effective indicator in mild patients, and early use of interferon has a good antiviral therapeutic effect.

Introduction

SARS-CoV-2 is the known coronavirus after severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV). It was first identified in late December 2019 and causes severe respiratory syndrome in humans^{1,2}. The disease caused by SARS-CoV-2 has been recently named COVID-19 by the World Health Organization (WHO). Globally, as of August 2020, there have been 24,854,140 confirmed cases of COVID-19, including 838,924 deaths. According to the epidemiological statistics, most of the COVID-19 patients (about 80%) belong to the mild patients^{3,4}. The city of Shiyuan is located in Hubei Province, and most of the COVID-19 patients there had mild cases. For this reason, it is necessary to study the immunological characteristics of mild COVID-19 patients and find a suitable therapeutic strategy to prevent the transformation of mild to severe patients.

Inflammation is the body's first line of defense against viral infection. It involves activating both the innate and adaptive immune responses. However, excessive immune responses after infection, also called a cytokine storms, have been found to be associated with extreme levels of pro-inflammatory cytokines and widespread tissue damage.

Preliminary studies have shown that SARS-CoV-2 infection triggers a cytokine storm, and results in the increase of a variety of cytokines, including chemokine⁵⁻⁷. Chemokines are low-molecular-weight proteins with powerful chemoattractant activity. They play a role in the immune cell recruitment during inflammation. Chemokines are classified according to their chemical structure, the C, CC CXC and CX3C families⁸. The binding of chemokines to their receptors is responsible for their chemoattractant ability.

The chemokine receptors are seven-transmembrane-spanning, G-protein-coupled receptors. They are expressed on leukocytes and endothelial cells, etc.⁹. Serum chemokine levels were found to be elevated in patients with COVID-19, and they were even higher in those who required ICU admission, suggesting a relationship with lung damage and disease severity¹⁰. However, it is not clear whether the concentration of chemokines is higher in mild COVID-19 patients, and the highly expressed chemokines can be used as a marker of the diagnosis and prognosis of mild COVID-19 patients.

Type I interferons have broad-spectrum antiviral activities against RNA viruses, which act by inducing an antiviral response and mediating adaptive immune response. Type I interferons include IFN- α and IFN- β ¹¹. Infection of cells with virus causes the activation of several cellular transcription factors, such as interferon regulatory factor 3/7 (IRF3/7) and NF- κ B, which activate the expression of a number of interferon-stimulated genes (ISGs) and exert antiviral effect^{12,13}. The downstream signaling pathway of the transcription factors can recruit and coordinate specific subsets of leukocytes, which is orchestrated primarily by chemokine secretion^{14,15}. This means that the secretion of chemokines may be related to the release of interferon, but the specific relationship between them in the process of SARS-CoV-2 infection has not been clearly established.

Clinically, Type I IFNs have already been approved for use in the treatment of certain cancers, autoimmune disorders, and viral infections¹⁶. Type I IFNs are currently in clinical trials to evaluate their ability to treat MERS-CoV and therefore have been proposed for the treatment of COVID-19, but there is currently no evidence from laboratory testing against SARS-CoV-2¹⁷.

In this study, we first demonstrated that COVID-19 patients are characterized by higher levels of chemokine. In particular, MCP-1 has shown higher expression in patients with mild cases of COVID-19 in Shiyuan City. The receptor of MCP-1, CCR2 was then found higher expression in the Peripheral blood mononuclear cells (PBMCs) from same mild COVID-19 patients. Finally, mild COVID-19 patients are found to have lower levels of IFN- β in the serum. The higher expression of MCP-1 in mild COVID-19 patients is correlated with the inhibition of IRF3. Our results suggested that MCP-1 may be an effective indicator in mild patients, and interferon was a good antiviral therapeutic agent for mild COVID-19 diseases.

Results

Mild COVID-19 patients have higher levels of MCP-1

According to the basic information of severe COVID-19 and mild COVID-19 patients in Table 1, it was suggested that there were no remarkable differences between them from hospital time and some complications. In order to further clarify the different role of chemokine in severe and mild COVID-19 patients, we detect three chemokine (including MCP-1, IP-10, and IL-8) in the serum among either severe or mild COVID-19 patients and healthy controls. It was demonstrated that MCP-1 upregulation was observed in almost all COVID-19 patients no matter what is severe or mild cases (Fig. 1A) ($P < 0.01$). In particular, MCP-1 has also shown higher expression in patients with mild cases of COVID-19. IP-10

showed upregulation in severe COVID-19 patients while no change in mild patients (Fig. 1B). Meanwhile, severe COVID-19 patients also showed the upregulation of IL-8 (Fig. 1C). Recent reports indicated that MCP-1, IP-10, and IL-8 levels were higher in COVID-19 patients and even higher among those admitted to ICU¹⁰. We also found that the level of expression of the three chemokine was increased in severe COVID-19 patients. It has been suggested that chemokines may play an important role in patients with severe COVID-19. The upregulation of MCP-1 in mild COVID-19 suggested that it may play the role in pathogenesis of mild diseases.

Table 1
Clinical characteristics of COVID-19 patients and healthy controls

Features	Severe COVID-19	Mild COVID-19	Healthy controls
Mean Age (year)	56.3	44.6	42
Gender (Female/Male)	30%	40%	40%
Average time of hospital (day)	28.4	23.4	-
Diabetes	20%	16.7%	-
Heart disease	20%	20%	-
Hypertension	40%	23.3%	-
Cancer	0%	0%	-
According to China National Health Commission Guidelines for Diagnosis and Treatment of SARS-CoV-2 infections (seventh version) issued by 2020.			

Table 2
The primer sequence

	Gene Name	Primer sequence
1	<i>CCR2</i>	Up: 5'- AAGAGGCATAGGGCAGTGAG - 3' Down: 5'- GGGATTGATGCAGCAGTGAG - 3'
2	<i>CXCR2</i>	Up: 5'- GCATCAGTGTGGACCGTTAC - 3' Down: 5'- GGCTGGGCTAACATTGGATG - 3'
3	<i>CXCR3</i>	Up: 5'- TGGTGGACATCCTCATGGAC - 3' Down: 5'- CAAGAGCAGCATCCACATCC - 3'
4	<i>IRF3</i>	Up: 5'- GACCCTCACGACCCACATAA - 3' Down: 5'- CAGAAGTACTGCCTCCACCA - 3'

CCR2 show greater expression in PBMC from mild COVID-19 patients

Chemokines, when combined with corresponding receptors, play a chemotactic role in immune cells. In order to further clarify the role of chemokine in mild COVID-19 diseases, we tested the receptors for MCP-1, IP-10, and IL-8, respectively. The expression level of the receptor of MCP-1, C-C motif receptor 2 (CCR2); the receptor of IP-10, chemokine (C-X-C motif) receptor 3 (CXCR3); and the receptor of IL-8, CXCR2 were assessed in the PBMC from mild COVID-19 patients and healthy controls. We observed the upregulation of CCR2 (Fig. 2A) ($P < 0.01$), while there was no change of CXCR3 (Fig. 2B) and CXCR2 (Fig. 2C) between mild COVID-19 patients and healthy controls. It was demonstrated that MCP-1 may participate in the pathogenesis of mild COVID-19 diseases by binding its receptor.

Higher expression of MCP-1 in mild COVID-19 patients is correlated with the inhibition of IFN signaling

Transcriptional activation of interferon regulator factors (IRFs) results in the launch of general antiviral programs. We then explored the expression levels of IRF3 in mild COVID-19 patients. The expression of IRF3, an important gene in the interferon signaling pathway, was down-regulated (Fig. 3A) ($P < 0.01$). Meanwhile, the downregulation of IFN- β was observed in mild COVID-19 patients (Fig. 3B) ($P < 0.01$). To clarify the relationship between serum MCP-1 and expression level of IRF3, we performed Spearman rank correlation analysis, the results showed that IRF3 downregulation was significantly negative correlated with MCP-1 (Fig. 3C) ($P < 0.01$, $r^2 = 0.861$). Our results suggest that MCP-1 may be an effective index for mild COVID-19 patients, and interferon was a good antiviral therapeutic agent for mild COVID-19 diseases.

Discussion

Previous studies have shown that elevated levels of pro-inflammatory cytokines, such as IFN- γ , TNF- α , IL-6 and IL-8, are associated with severe lung injury and adverse outcomes of SARS-CoV or MERS-CoV infection¹⁸⁻²⁰. It has also demonstrated that severe COVID-19 patients have higher concentrations of chemokine in the serum than mild cases, suggesting that the magnitude of cytokine storm is associated with the disease severity^{7,9}.

Most of COVID-19 patients in Shiyuan city are mild cases. In order to further study the level of chemokines in mild COVID-19 patients, we detected the level of chemokines in the serum of mild COVID-19 patients admitted to Xiyuan Hospital and Renmin Hospital in Shiyuan City. We selected monocyte chemokine, MCP-1, interferon induced protein 10, IP-10 and neutrophil chemokine, IL-8. MCP-1, this protein belongs to the C-C chemokine family and is a powerful monocyte chemotactic factor that is constitutively produced or induced by oxidative stress, cytokines, or growth factors. Monocytes and macrophages are the main source of MCP-1, which regulates the migration and infiltration of monocytes, memory T cells, and NK cells²¹. Huang et al. found that MCP-1 levels were higher in patients with COVID-19 and even higher among those admitted to ICU¹⁰. It has been reported that MCP-1 increases rapidly in the early acute

phase of infection and then progressively decreases with the advance of the disease²². Xiong et al. detected elevated levels of MCP-1 in the bronchoalveolar lavage fluid of patients with COVID-19 and found it to be associated with the pathogeny of the virus²³. Elevated levels of MCP-1 have also been detected in the lung tissue of patients infected with SARS-CoV-2²⁴. IP-10 was initially identified as a chemokine whose secretion is induced by IFN- γ . IP-10 is secreted by neutrophils, endothelial cells, keratinocytes, fibroblasts, dendritic cells, astrocytes, and hepatocytes. Through its binding to chemokine receptor 3 (CXCR3), it regulates immune system responses by activating and recruiting leukocytes, including T cells, monocytes, and NK cells. Therefore, IP-10 and CXCR3 play a key role in recruiting leukocytes to inflamed tissues and in perpetuating inflammation, thereby making a major contribution to tissue damage²⁵. Serum IP-10 levels were found to be elevated in patients with COVID-19 and they have been found to be even higher in those who required ICU admission, suggesting a relationship with lung damage and disease severity¹⁰. Liu et al. associated elevated serum IP-10 levels with a higher viral load and greater lung damage in patients with SARS-CoV-2²⁶. Recent reports suggested that the expression level of IL-8 was higher in patients with severe COVID-19¹⁰. Our results also proved that MCP-1, IP-10 and IL-8 were up-regulated in severe COVID-19 patients (Fig. 1). It has been suggested that chemokines may play an important role in patients with severe COVID-19. In particular, MCP-1 has shown higher expression in patients with mild cases of COVID-19 (Fig. 1A). IP-10 and IL-8 showed upregulation in severe COVID-19 patients while no change in mild patients (Fig. 1B, 1C).

In order to further clarify the role of chemokine in mild COVID-19 diseases, we tested the receptors for MCP-1, IP-10, and IL-8²⁷, respectively. Between mild COVID-19 patients and healthy controls, CCR2 was up-regulated (Fig. 2A) ($p < 0.01$), while CXCR3 (Fig. 2B) and CXCR2 (Fig. 2C) did not change. It was demonstrated that MCP-1 may participate in the pathogenesis of mild COVID-19 diseases by binding its receptor.

Engagement of virus-specific RNA structures culminates in oligomerization of these receptors and activation of downstream transcription factors, most notably interferon regulator factors (IRFs) and nuclear factor kB (NF-kB). We found that, in mild COVID-19 patients with higher level of MCP-1, the expression of IRF3, an important gene in the interferon signaling pathway, was down-regulated (Fig. 3A). IFN- β levels in serum of peripheral blood from COVID-19 patients were lower than that of healthy controls (Fig. 3B). Meanwhile, IRF3 downregulation was significantly negative correlated with MCP-1 (Fig. 3C). Other studies have already established that SARS-CoV-2 has greater sensitivity to IFN-I than SARS-CoV²⁸. In these studies, pre-treatment with IFN- α or IFN- β drastically reduced viral titers. These findings suggest that IFN-I may be effective as a prophylactic agent or an early treatment option for SARS-CoV-2. However, delayed IFN administration was of no benefit over a placebo²⁹. Channappavanar et al. showed that delayed IFN-I expression can be detrimental in mice in the context of SARS-CoV-1 infection³⁰. The timing of interferon exposition may be critical for controlling the virus and avoiding immunopathogenesis. Early administration of IFN was slightly beneficial to reducing viral load and improving clinical outcome in COVID-19 patients.

Conclusions

In summary, our results suggested that inhibition of interferon signaling pathway may be an effective mechanism of viral immune escape, and interferon supplementation may be a useful therapeutic strategy for mild COVID-19 patients.

Abbreviations

SARS-CoV: severe acute respiratory syndrome coronavirus; MERS-CoV: Middle East respiratory syndrome coronavirus; WHO: World Health Organization; IRF3/7: interferon regulatory factor 3/7; PBMCs: Peripheral blood mononuclear cells; ELISA: Enzyme linked immunosorbent assay; MCP-1: monocyte chemotactic protein 1; IP-10, Interferon-inducible protein-10; CCR2: C-C motif receptor 2; CXCR: chemokine (C-X-C motif) receptor.

Declarations

Ethics approval and consent to participate: The study protocol received approval from the Clinical Ethics Committee of Hubei University of Medicine (2020-TH-017). All individuals gave their informed consent to participate.

Consent for publication: Not applicable.

Availability of data and materials: All data generated or analysed during this study are included in this published article.

Competing interests: The authors declare that they have no competing interests.

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Author Contributions: Conceived and designed the experiments: XX YW. Performed the experiments: XX, YG, MZ. Analyzed the data: XX BD, YW. Contributed reagents/materials/analysis tools: YW, GL. Wrote the paper: XX, BD.

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Materials And Methods

Subjects.

The diagnose of COVID-19 was graded according to China National Health Commission Guidelines for Diagnosis and Treatment of SARS-CoV-2 infection (seventh version). 10 severe and 30 mild COVID-19 patients were recruited from Xiyuan Hospital and Renmin Hospital, Shiyuan City and the study protocol received approval from the Clinical Ethics Committee of Hubei University of Medicine (2020-TH-017). Another 10 healthy subjects were recruited as a control group. All individuals gave their informed consent to participate. The basic information of COVID-19 patients and healthy controls is listed in Table 1.

Isolation of Peripheral blood mononuclear cells (PBMCs)

PBMCs were isolated through density-gradient centrifugation using Ficoll-Paque (Sigma, 17144002) from peripheral blood samples of the participants in this study. Isolated PBMCs were cultured in the presence of RPMI 1640 medium (supplemented with 10% fetal bovine serum) at 37°C in an incubator with 5% CO₂.

Real-time PCR

After RNA was isolated from PBMCs, it was reverse transcribed into cDNA. Real-time quantitative PCR was performed to quantify chemokine receptors and transcript factor and GAPDH levels by using SYBR Premix Ex Taq (TaKaRa, RR820A) in the Bio-rad CFX manager 3.1 software. The primer sequences were listed in Table 2.

Enzyme linked immunosorbent assay (ELISA)

The chemokine monocyte chemotactic protein 1 (MCP-1), Interferon-inducible protein-10 (IP-10), Interleukin 8 (IL-8) and interferon β (IFN- β) in the serum of COVID-19 patients and healthy controls were determined by ELISA kit according to the manufacturer's instruction (1117392, 1110802, 1117452, Dakewei.Inc, KE00187, Sanying. Inc.).

Statistical analysis

Analysis of Variance (ANOVA) tests were used to compare the plasma chemokine levels among the COVID-19 patients and healthy control groups. The Spearman rank correlation coefficient was used for linear correlation analysis between the expression level of plasma chemokine and IRF3. All data were analyzed using SPSS version 19.0 and GraphPad 5.0 software. *P* value less than 0.05 was considered statistically significant.

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Figures

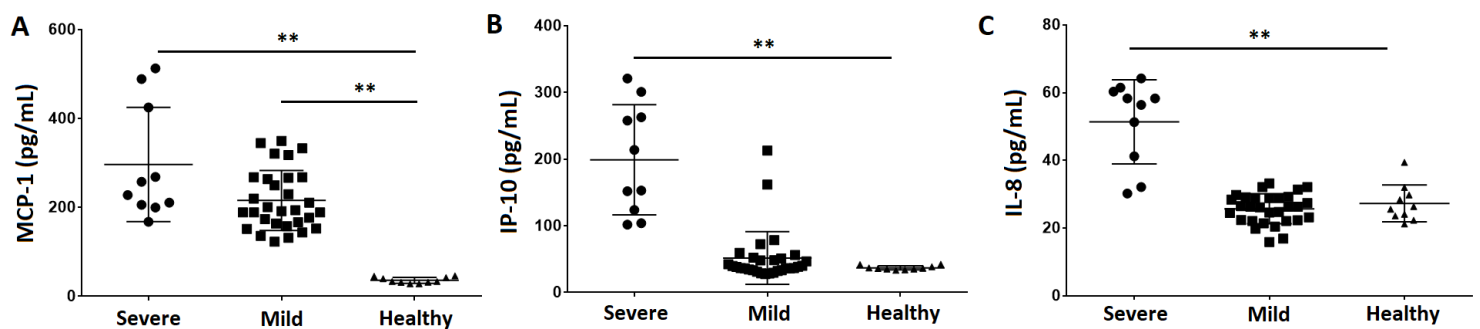


Figure 1

Mild COVID-19 patients have higher levels of MCP-1 (A). MCP-1 levels in serum of peripheral blood from COVID-19 patients were higher than that of healthy controls. (B) IP-10 and (C) IL-8 levels in serum of peripheral blood from severe COVID-19 patients were higher than that of the mild COVID-19 patients and healthy controls. The chemokine levels in serum of study subjects were detected by ELISA according as the manufacturer's instruction. **denote that $p < 0.01$.

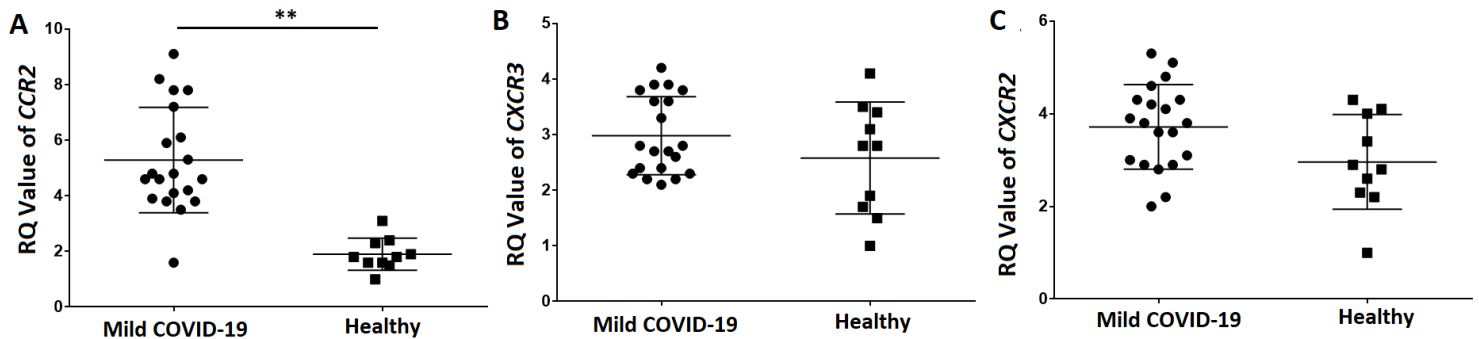


Figure 2

CCR2 show greater expression in PBMC from mild COVID-19 patients (A) The expression level of CCR2 is higher expressed in PBMC from mild COVID-19 patients. (B) CXCR2 and (C) CXCR3 have no difference between mild COVID-19 patients and healthy controls. After the RNA of PBMCs from mild COVID-19 patients and healthy controls, it was reverse transcribed into cDNA. Real-time quantitative PCR was performed to quantify chemokine receptors and GAPDH levels by using SYBR Premix Ex Taq. **denote that $p < 0.01$.

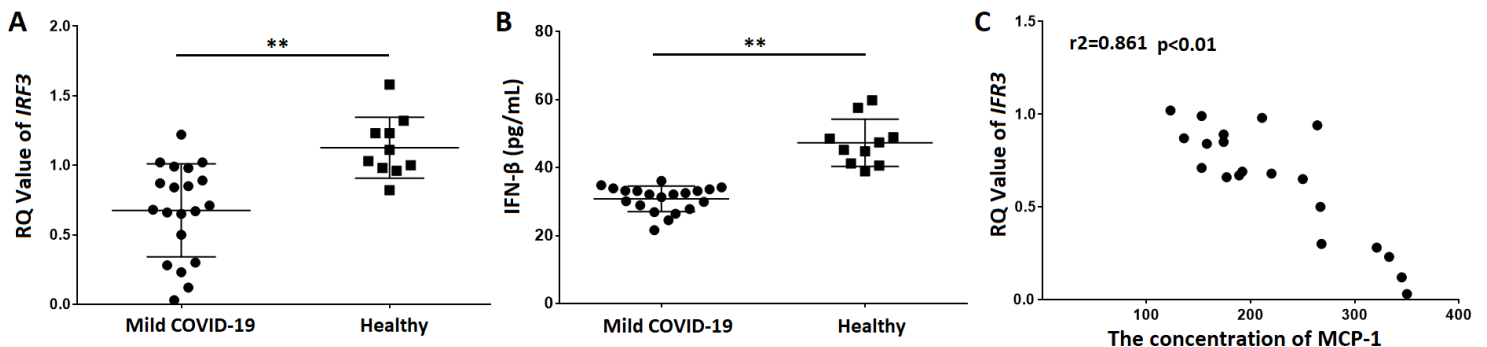


Figure 3

Higher expression of MCP-1 in mild COVID-19 patients is correlated with the inhibition of IFN signaling (A) IFR3 expression level has significant downregulation in mild COVID-19 patients. The RNA sample of PBMCs from 20 mild COVID-19 patients and 10 healthy controls was extracted and IFR3 were detected by real-time PCR. (B) IFN- β levels in serum of peripheral blood from COVID-19 patients were lower than that of healthy controls. The cytokine levels in serum of study subjects were detected by ELISA according as the manufacturer's instruction. (C) Spearman rank correlation analysis was performed to analyze the serum MCP-1 and the expression level of IFR3, it was showed that the expression IFR3 was significantly negative correlated with the concentration of MCP-1. **denote that $p < 0.01$.