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Effect of MCP-1 and CCR2 Serum Levels on COVID-19 Severity

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ABSTRACT

Objective: Approximately 80% of people with coronavirus disease 2019 (COVID-19) are asymptomatic, and only a small proportion of cases show serious consequences leading to hospitalization. The interplay between chemokines and their receptors can affect the severity of several infectious diseases, such as severe acute respiratory syndrome and Middle East Respiratory Syndrome. The interplay of monocyte chemoattractant protein-1 (MCP-1) with its receptor C-C motif chemokine receptor 2 (CCR2) may affect the pathogenesis of COVID-19 by functioning in the dispatch of lymphocytes and monocytes/macrophages to the infection site.

Materials and Methods: The serum MCP-1 and CCR2 concentrations were measured using the enzyme-linked immunosorbent assay (ELISA) in 49 asymptomatic, 50 severe, and 57 critical COVID-19 cases.

Results: Serum MCP-1 levels were considerably higher in critical cases than in cases in the other two groups, suggesting an increased risk for disease severity ($p = 0.008$; $p = 0.01$, respectively). Serum CCR2 levels were significantly higher in asymptomatic cases than in critical cases suggesting a protective role against disease severity ($p = 0.001$).

Conclusion: MCP-1 and CCR2 may be candidate biomarkers for the prediction of disease severity. Therefore, by measuring serum levels of MCP-1 and CCR2 early, the disease course can be predicted , and necessary precautions can be taken before the disease becomes severe.

Keywords: MCP-1, CCR2, COVID-19 severity, risk factors, candidate biomarkers

INTRODUCTION

The coronavirus disease 2019 (COVID-19) is a multifaceted respiratory ailment, with initial symptoms ranging from fever and dry cough to fatigue (1, 2). In confirmed cases, less common yet noteworthy symptoms include headaches, dizziness, abdominal discomfort, and gastrointestinal distress (3). In addition, approximately 80% of individuals with infection remain asymptomatic, whereas only a fraction of cases progress to a severe state necessitating hospitalization (4). Older people, men, smokers, and people with chronic diseases have more severe COVID-19 (5, 6). COVID-19 may cause pneumonia, liver injury, cardiac injury, sepsis, and death (7).

The course of COVID-19 not only depends on viral infection, but the host immune response also determines the disease outcome. The above normal inflammatory responses in cases that have advanced to the pneumonia stage increased the release of proinflammatory cytokines and chemokines, known as the "cytokine storm," which is more lethal than the viral infection itself and causes widespread

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alveolar destruction, fibrosis, worsening respiratory failure, and multiorgan dysfunction (8). Central to this cytokine storm is the heightened release of Interleukin (IL)-6, and high levels of IL-10 and IL-1 receptor antagonists also correlated with disease severity (9, 10).

Levels of chemokines, such as C-X-C motif chemokine ligand 10 (CXCL10) and monocyte chemoattractant protein-1 (MCP1), are markedly high in severe cases, contributing to the exacerbated inflammatory response (11). Notably, specific chemokine levels correlate positively with viral load exclusively in severe cases (12). Given their role in attracting leukocytes to infection sites, regulating their function holds significant promise in mitigating inflammation above normal levels in patients with COVID-19 (13).

By binding to its receptor C-C motif chemokine receptor 2 (CCR2), MCP-1 orchestrates the recruitment of monocytes and basophils, influencing processes such as inflammation, angiogenesis, and coagulation (14). This interaction may play a pivotal role in the pathogenesis of viral infections by facilitating the dispatch of lymphocytes and monocytes/macrophages to the infection site (15). Although MCP-1 has been proposed as a disease biomarker (16), unraveling the precise role of the MCP-1/CCR2 pathway in COVID-19 remains imperative. This study endeavors to elucidate the association between MCP-1 and CCR2 serum levels in COVID-19 to pinpoint potential biomarkers for disease severity and prognosis.

Table 1. Demographic features and the clinical characteristics of patients with COVID-19.

	Grup A (Asymptomatic) (n=49)	Grup B (Severe) (n=50)	Grup C (Critical) (n=57)	p Values		
Variables				**Grup A vs B**	**Grup A vs C**	**Grup B vs C**
Age (Mean ± SD, years)	45.6 ± 13.9	52.7 ± 13.5	59.3 ± 15.5	0.001	0.001	0.396
Gender						
Female, n (%)	29 (59.2%)	21 (42.0%)	20 (35.1%)	0.087	0.013	0.483
Male, n (%)	20 (40.8%)	29 (58.0%)	37 (64.9%)			
Comorbidity						
Yes, n (%)	32 (65.3%)	37 (74.0%)	39 (68.4%)	0.470	0.894	0.673
No, n (%)	17 (34.7%)	13 (26.0%)	18 (31.6%)			
Diabetes						
Yes, n (%)	8 (16.3%)	14 (28.0%)	13 (22.8%)	0.248	0.555	0.693
No, n (%)	41 (83.7%)	36 (72.0%)	44 (77.2%)			
Hypertension						
Yes, n (%)	6 (12.2%)	12 (24.0%)	16 (28.1%)	0.209	0.077	0.796
No, n (%)	43 (87.8%)	38 (76.0%)	41 (71.9%)			
Blood Test Results, median (IQR)						
D-dimer (µg/mL)	0.4 (0.6)	0.5 (1.2)	3.3 (6.7)	0.983	0.001	0.001
Hemoglobin (g/dL)	13.2 (13.1)	12.9 (12.9)	13.3 (12.2)	0.189	0.947	0.344
Lymphocytes ($x10^9$/L)	1.4 (1.5)	1 (1.2)	0.7 (3.6)	0.874	0.453	0.713
WBC ($x10^9$/L)	5.8 (6.4)	6.6 (9.3)	10.9 (11.2)	0.437	0.0001	0.001
Platelets ($x10^9$/L)	205.5 (218.7)	222.5 (229.5)	250 (238.5)	0.798	0.999	0.761
CRP (mg/L)	24.3 (34)	45.5 (70.1)	100.3 (134.2)	0.075	0.001	0.0001
Ferritin (mg/mL)	148.4 (186.4)	492.6 (664.3)	887.4 (1719.7)	0.001	0.0001	0.001

MATERIALS AND METHODS

Patients

In this study, the case group comprised 49 patients with asymptomatic COVID-19 who required only home quarantine, 50 patients who were hospitalized, and 57 patients with critical conditions who had severe COVID-19 and needed intensive care. Real-time reverse transcription polymerase chain reaction (RT-PCR) of viral nucleic acid was accepted as the reference standard in the diagnosis of COVID-19. Chest computed tomography was used to detect patients with COVID-19 pneumonia. These patients originated from Turkiye and were enrolled in the study to measure the serum MCP-1 and CCR2 levels. Participants were selected from cases coming to Kanuni Sultan Suleyman Training and Research Hospital between June 2022 and September 2022 and volunteered to participate in the study randomly.

Measurements of Serum MCP-1 and CCR2 Levels

Blood samples were taken from patients with asymptomatic COVID-19 when they first applied to the outpatient clinic, patients with severe disease while they were hospitalized in the service, and patients with critical disease while they were in the intensive care unit. The enzyme-linked immunosorbent assay (ELISA) was conducted to determine MCP-1 and CCR2 serum levels, which are taken from patients' whole blood. The analytical measurement ranges were 62.5–4000 pg/mL for MCP-1 and 0.16–10 ng/mL for CCR2.

Statistical Analyses

The obtained data were statistically analyzed using the IBM SPSS Statistics version 21.0. For the correlation of countable values with each other, r and p values were given by looking at the correlation. A normality test was performed for the values. The Kolmogorov–Smirnov test was used. Spearman's rho correlation was applied because MCP-1 and CCR2 values of the variables showed no normal distribution. The Kruskal–Wallis test and then the post-hoc test were used for crosschecking the MCP-1 and CCR2 values among the three groups. A multivariate logistic test was applied for the effect of independent variables (age, sex, biochemical parameters, diabetes, hypertension, and comorbidity) on MCP-1 and CCR2 between groups. A sample size was calculated with a two-sided confidence interval. Considering a 0.80 power and a 0.05 error, 43 patients were needed for a standard deviation of 0.2. Considering a possible loss of 15% during data collection, at least 150 patients were included. p significance limit was accepted as less than 0.05.

RESULTS

In the study group, participants in the critical COVID-19 group (age range: 28–91; mean age: 59.3 ± 15.5 years; female/male: 20/37) were statistically significantly older than those in the asymptomatic group (age range: 21–75; mean age: 45.6 ± 13.9 years; female/male: 29/20) and severe group (age range: 18–79; mean age: 52.7 ± 13.5 years; female/male: 21/29), and the number of male patients was greater as noted in previous studies. No statistically significant relationship was found between diabetes, hypertension, comorbidities, and disease severity in the study group. In addition, D-dimer, C-reactive protein (CRP), and ferritin levels in the study group were statistically significantly higher than those in the asymptomatic to the critical group. Table 1 demonstrates all parameters related to the aim of this study.

Table 2 shows the distribution of the serum MCP-1 and CCR2 levels in the three groups. Serum MCP-1 levels were considerably higher in the critical group than those in the other two groups, suggesting an increased risk for severe disease ($p = 0.008$; $p = 0.01$, respectively). Serum CCR2 levels were significantly higher in the asymptomatic group than in the critical group, suggesting a protective role against severe disease ($p = 0.001$).

In addition, the relevance between clinical parameters and serum MCP-1 and CCR2 levels was examined in the study group. An inverse relationship (negative correlation) was found between serum MCP-1 levels and platelet count in the asymptomatic and critical groups ($r = -0.356$, $p = 0.015$; $r = -0.361$, $p = 0.013$, respectively). An inverse relationship was found between serum CCR2 and CRP levels in the asymptomatic group ($r = -0.295$, $p = 0.044$). A linear relationship (positive correlation) was found between MCP-1 serum, ferritin, D-dimer, and white blood cell count as expected considering the literature on critical COVID-19 ($r = 0.279$, $p = 0.037$; $r = 0.349$, $p = 0.009$; and $r = 0.295$, $p = 0.029$, respectively). A linear

Table 2. MCP-1 and CCR2 levels in asymptomatic, severe and critical COVID-19 patients.

	Grup A (Asypmtomatic) (n=49)	Grup B (Severe) (n=50)	Grup C (Critical) (n=57)	p Values		
Variables				**Grup A vs B**	**Grup A vs C**	**Grup B vs C**
MCP-1 (pg/mL), median (IQR)	315.2 (149.9)	267.7 (262.9)	549.6 (954.8)	0.999	0.008	0.01
CCR2 (ng/mL), median (IQR)	8.8 (3.5)	6.6 (6.3)	5.6 (4.2)	0.082	0.001	0.431

relationship was noted between serum CCR2 levels and age in the critical group ($r = 0.307$, $p = 0.02$). Moreover, an inverse relationship was found between serum CCR2 and lymphocyte levels in the critical group ($r = -0.337$, $p = 0.013$). Serum MCP-1 and CCR2 levels were not related to any of the clinical parameters in the severe group (data not shown).

Practical Implications of the Study's Findings

Clinicians can more closely monitor patients who they consider at risk based on serum MCP-1 and CCR2 levels and can take precautions in advance in patients who they predict will have increased inflammatory response. They can develop treatments to reduce inflammatory responses in these patients. Researchers can investigate different treatment possibilities and discover new drugs that affect the MCP-1 and CCR2 pathways.

DISCUSSION

The interplay between chemokines and their corresponding receptors holds implications for susceptibility to various diseases, including atherosclerosis, multiple sclerosis, and colitis (17, 18, 19). These molecules have also been implicated in infectious diseases such as severe acute respiratory syndrome (SARS) and Middle East Respiratory Syndrome (MERS) (20). Notably, deficiencies in chemokine receptors such as CCR1, CCR2, and CCR5 have been linked to severe illness and fatality because of diminished recruitment of infection-fighting cells into the lungs (21).

MCP-1 and CXCL10 suppress the proliferation of cells that cause lymphopenia in SARS and MERS, and their plasma levels positively correlated with mortality in MERS-CoV infection (22, 23). Thus, chemokines may also affect ones susceptibility to COVID-19, and the severity of COVID-19 can be stratified by measuring serum chemokine levels.

In this study, serum MCP-1 levels were significantly higher in the critical group than in the asymptomatic and severe groups. This observation is consistent with prior findings indicating a proportional increase in MCP-1 levels with the COVID-19 severity (24). Anderberg et al. similarly established that high serum MCP-1 levels were related to respiratory failure and mortality in critical COVID-19 (25). Genetic studies further supported our findings, with polymorphisms associated with high MCP-1 levels aligning with disease severity (26). In addition, a linear relationship between serum MCP-1 levels and D-dimer levels was noted in the critical group, suggesting interplay between inflammation and coagulation in the progression of COVID-19 (27).

Interestingly, CCR2 levels were considerably higher in the asymptomatic group than in the critical group. This implies that high serum CCR2 levels may confer protection against the severe effects of COVID-19. Previous studies have indicated the upregulation of CCR2, alongside CXCR3, in pulmonary responses to MERS infection (28). Mouse models infected with SARS-CoV have also demonstrated deficiencies in CCR2 and CCR5, which increases the mortality risk (29). Genetic analyses have further identified CCR2, CCR3, and CXCR6 as potential causal genes for COVID-19 severity, emphasizing the critical role of chemokine receptors in disease progression (30).

Potential limitations of this study included the relatively small sample size, recruitment of patients from a single center, and short sampling window (up to 10 days from intubation) during a prolonged course of an acute illness.

This study confirmed that MCP-1 could be used to predict COVID-19 severity and showed that CCR2 may be a candidate biomarker for the prediction of disease severity. Considering that COVID-19 severity is affected by similar factors in studies conducted in other countries, our findings may be valid in other populations. Therefore, by measuring serum MCP-1 and CCR2 levels early, the disease course can be predicted, and necessary precautions can be taken before the disease becomes severe. In addition, by reducing the response to MCP-1 by using certain antagonists for CCR2 receptor blockade, increased inflammation and disease exacerbation can be prevented.

Ethics Committee Approval: Ethics committee approval was obtained for this study from the Kanuni Sultan Suleyman Training and Research Hospital (Date: 22.06.2022, No: 150).

Informed Consent: Signed consent was obtained from the participants.

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