Short Communication

Association of Monocyte–Macrophage Chemotactic Receptor 2 Gene Polymorphisms with Covid-19 Infection in Karbala, Iraq

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Abstract

Background: Between 2019 and early 2020, the appearance of an unusual strain related to the coronavirus caused a worldwide pandemic. During this infection, the immune response by different types of cells can trigger the creation of monocyte–macrophage chemotactic receptors to facilitate monocyte infiltrates CCL2/CC chemokine receptor 2 (CCR2) pathway. **Objectives:** This research aims to investigate the relationship between polymorphisms and serum levels of CCR2 in patients infected with COVID-19 and its association with the development of symptoms. **Materials and Methods:** A total of 150 SARS-CoV-2 infected patients and 50 healthy controls participated in the study. Real time PCR for viral swabbing and strip antigens confirmed the diagnosis. Participants received medical care at IMAM Al-Hussein Medical City from March to July 2022; the mild group included 36 men and 14 women. Nonetheless, 31 men and 19 women were in moderate and severe groups, and the average age was (44.04, 62.16, and 61.14 years) for the three groups. Then, the manufacturer's instructions for CCR2 level parameters used sandwich ELISA and allele-specific primers assay for CCR2 receptor (rs1799864) polymorphisms. **Results:** A study of CCR2 in human serum found a positive correlation and significance ($r = 0.564$, $p \leq 0.001$) with COVID-19 symptoms. However, CCR2 polymorphisms in mild, moderate, and severe groups were nonstatistical for SNP (rs1799864), which raised the odds ratio with increased symptoms; Heterozygote mutant GA allele (rs1799864) was more frequent and increased odds ratio in patients' groups; therefore, it was considered a risk factors development of symptoms COVID-19. **Conclusion:** Both parameter studies are the polymorphisms and level CCR2 raised in individuals suffering from COVID-19.

Keywords: 2-CoV-macrophage chemotactic receptor 2, monocyte-polymorphisms, SARS

Introduction

A new strain of the coronavirus appeared in Wuhan, a Chinese city, where the virus spread to all parts of the world. However, some local studies found that the source of infection came from a market selling fish and wild animals; this confirms the hypothesis of transmission of the virus from animal to human, as well as the potential for virus severe acute respiratory syndrome coronavirus (SARS-CoV-2) transference from someone to another, in both ways during direct contact or by droplets via the surroundings air or touching surfaces contaminated with the virus. In February 2020, the World Health Organization launched a new epidemic, "COVID-19 disease."[1-3]

This virus can cause pulmonary infection, and individuals tend to be asymptomatic or symptomatic.[4] Knowledge regarding the clinical characteristics of COVID-19 patients in Iraq was emphasized as crucial.[5]

Chemokine receptors can elicit distinct, tissue-specific cellular movement patterns. The presence of disruptions in chemokine quantities and their expression of receptors for chemokine has been observed in cases of

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severe COVID-19, underscoring their significance in immunopathology.[6]

In inflammation locations, many different kinds of cells can trigger the creation of CC chemokine receptor 2 (CCR2). However, it is mainly produced via particular cell types, likely monocytes, dendritic cells, macrophages, natural killer cells, and T lymphocytes.[7] Regarding the crucial role of the CCL2/CCR2 pathway for encouraging monocytes infiltrate through the respiratory region along with eventual tissue harm, it appears that targeting the CCL2/CCR2 pathway holds promise as an expected medical therapy for controlling as well as immunomodulating individuals afflicted.[8] The CCR2 is indispensable in recruiting immunological and non-immunological cells during pathological circumstances. It is a receptor for monocyte chemoattractant protein-1 (MCP-1).[9]

The genetic intermediaries CCR2 have been identified as genetically associated with severe illnesses triggered by novel coronavirus through an impartial investigation of the molecular strategies underlying this phenotype. The variants of serious COVID-19 correlate with CCR2 mutations that predict elevated levels of CCR2 within pulmonary tissue.[10] Excessive activation within this CCL2/CCR2 axis triggers excessive inflammation and subsequent organ damage.[11]

CCR2 V64I mutations can impact the risk of illness throughout Asian countries, although there is not any information about CCR2 polymorphisms regarding COVID-19 conditions.[12]

The current study aims to evaluate the relationship between some parameters in patients infected with COVID-19 and its association with development symptoms by studying polymorphisms with serum level of immunological receptors (monocyte–macrophage chemotactic receptor2 (CCR2).

Materials and Methods

The studied groups and samples

A cross sectional study was carried out at IMAM Al-Hussein Medical City from March to July 2022, and it included 150 COVID-19 patients of both sexes who were chosen randomly in addition to the control group, which included 50 healthy persons. Before participating in this study, each subject gave permission, and we performed a detailed questionnaire such as name, age, sex, onset of disease, and other diseases for all participants; then, disposable syringes were used to collect 5 mL venous blood samples from all groups.

Exclusion criteria

The study excluded persons infected by other diseases, individuals with chronic or immune disorders such as diabetes, pregnant women, cancer and kidney disease, smokers with systemic immune disease and thyroid gland diseases and those on long-term oral corticosteroid anti-inflammatory therapy were also excluded.

Laboratory investigations

The monocyte–macrophage chemotactic receptor 2 ELISA Kit can be accurately and quantitatively detected in blood using this Sandwich kit. The testing followed the manufacturer's directions (BT lab company), and allele-specific primers performed polymorphisms.

Statistical analysis

Data were collected, summarized, analyzed, and presented using the Statistical Package for the Sciences of Society (SPSS) version 22 and GraphPad Prime 8. The more quantitative parameters were represented using numerical values along with percentages, whereas quantitative (numeric) factors were first evaluated for abnormality distribution using the Kruskal–Wallis test.

Ethics approval and consent to participate

Written approval had been gained from the Ethics Committee of the Ministry of Health Karbala Health Department Imam Al-Hussein Medical City Ethics (no. 43 in February 22, 2022). Study data/information was used for research purposes only. Information consent and verbal from every participant were taken.

Result

Correlation between CCR2 and COVID-19 infection

The serum level of CCR2 in patients and control individuals is illustrated in Figure 1. Serum levels of CCR2 included means (94.27, 157.8, 251.5, and 269.0 ng/mL) for control, mild, moderate, and severe, respectively;

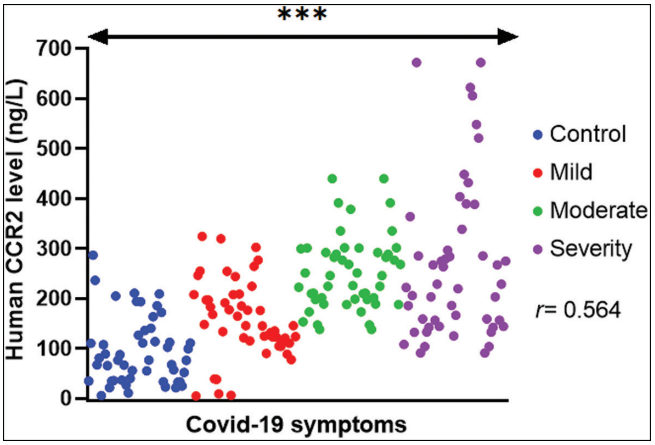

Figure 1: The CCR2 level in studied groups, a non-significance value was indicated as **ns** between the groups. The level of probability was indicated as $P < 0.05$. Data was presented as mean, statistical test: Kruskal Wallis test, $n = 200$

Table 1: Frequency distribution of genotypes according to groups based on Hardy–Weinberg equilibrium

Genotype CCR2 rs1799864	Control $n = 50$		Mild $n = 50$		Moderate $n = 50$		Severe $n = 50$	
	Observed	Expected	Observed	Expected	Observed	Expected	Observed	Expected
GG	5	9.75	8	9.75	12	9.75	14	9.75
GA	45	40	42	40	38	40	35	40
AA	0	0.25	0	0.25	0	0.25	1	0.25
χ^2	3.189		0.664		0.869		4.728	
P	0.203		0.717		0.6475		0.09	

n = cases; χ^2 = Qi square; * = significant at $P < 0.05$

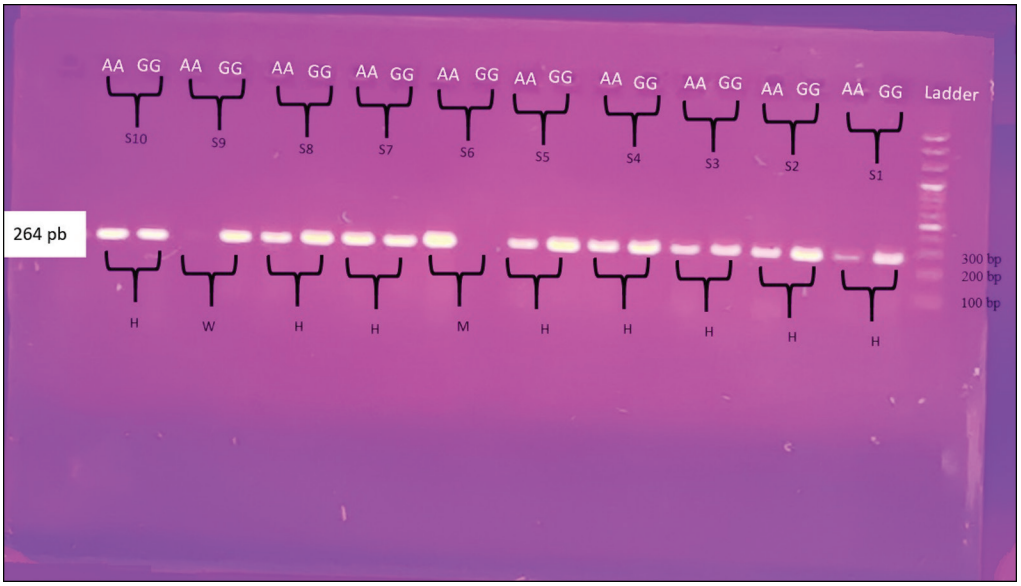

Figure 2: Ethidium bromide-stained agarose gel showing, the allele specific primer -PCR products for CCR2 (rs1799864) polymorphism, (GG genotype), (GA genotype) and (AA genotype)

there was a significant difference in the distribution of patients and control subjects according to symptoms with a strong positive relationship ($r = 0.564$**, $p \leq 0.001$).

Genotype study of CCR2 gene polymorphism

The frequency distribution of genotypes according to groups based on Hardy–Weinberg equilibrium is shown in Table 1. There was a non-significant statistical in the frequency distribution of observed and expected count of CCR2 genotypes in all patients' groups compared with the control group, This experiment was conducted based on allele-specific primer using agarose gel with a 1000 bp ladder and ethidium bromide dye with a PCR product size 264, as shown in Figure 2, Although there were non-significant statistically; during the current study, it was noted that the heterozygote mutant allele is a risk factor for all patients as showed in Figures 3 and 4.

Discussion

Correlation between CCR2 and COVID-19 infection

Furthermore, the binding of CCR2 through MCP-1 facilitates the immune system's natural reaction by attracting monocytes towards inflammation areas.[13] The route between CCL2 and CCR2 is essential in the migration of lymphocytes and monocytes/macrophages, which can be linked to certain diseases, including infections attributable to viruses.[7] The upregulation of the CCR2 within peripheral B cells and T cells during infectious illnesses indicates a significant role of CCR2 in regulating the immune reaction.[14] Furthermore, the suppression of the CCR2 pathway has been shown to decrease the concentrations of storm-related cytokines mediators, such as interleukin-6, circulating tumor necrosis factor α, interferon-γ, as well as macrophage inflammatory processes, thereby impacting the immune system's response. The findings emphasized the significance of CCR2 about the circumstances of coronavirus infections.[15] According to Vanderheiden *et al.*,[16] it has been demonstrated that the parenchymal monocyte-derived cells exhibit a defensive role against SARS-CoV-2. This is evidenced by the fact that animals lacking CCR2 displayed higher concentrations of viral particles in the respiratory tract, increased virus diffusion inside the lungs, and heightened cytokines associated with inflammation responses.

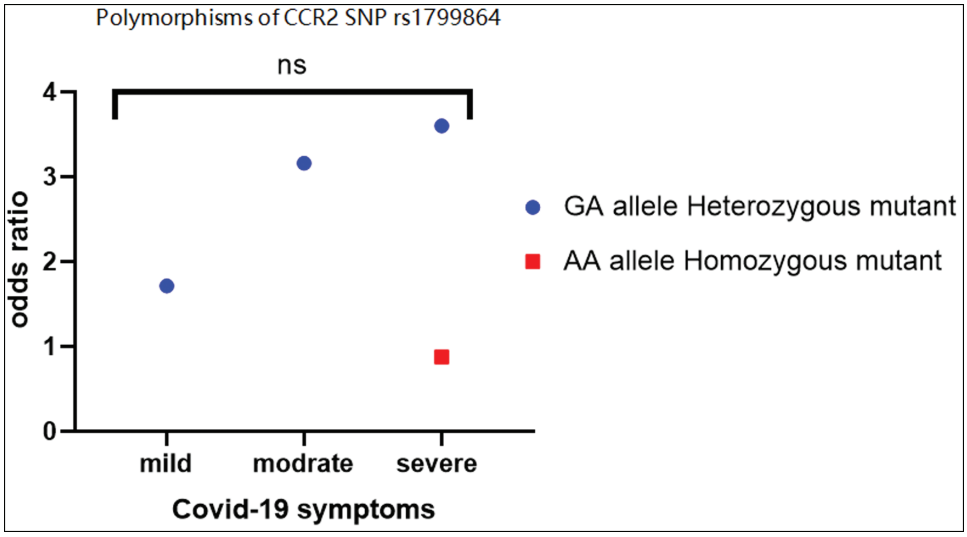

Figure 3: Polymorphisms of CCR2 SNP rs1799864 in studied groups, a non-significance value was indicated as **ns** between the groups. The level of probability was indicated as $P < 0.05$. Data was presented as mean, statistical test: Kruskal Wallis test, $n = 200$

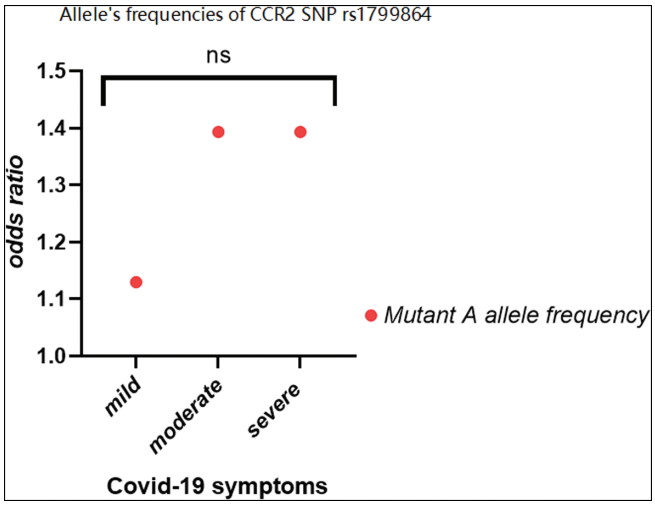

Figure 4: Allele frequencies of CCR2 SNP rs1799864 in studied groups, a non-significance value was indicated as **ns** between the groups. The level of probability was indicated as $P < 0.05$. Data was presented as mean, statistical test: Kruskal Wallis test, $n = 200$

Genotype study of CCR2 gene polymorphism

The occurrence of CCR2 genotyping (rs1799864) and allele frequencies are compared between the COVID-19 patients and control group in the Figures 3 and 4. Genotype GG allele, indeed wild type, considered reference comparison with the mild, moderate, and severe groups.

While genotype G/A, considered the heterozygous mutant type, was highly frequent in mild, moderate, and severe groups (P = 0.3762, 0.0438; 0.0240) respectively. Therefore, it was an odds ratio of (1.7143, 3.1622; 3.60) receptively. Consequently, it was regarded considered a risk factor for those with three patients' groups. Genotype A/A homozygous mutant type occurred in severe groups and was absent in others, so it is considered a lower risk factor than the heterozygous mutant type. Allele A was more frequent in patients with mild (P = 0.6688), moderate and severe groups (P = 0.2507); it was with an odds ratio of the mild group (1.1299) while for both moderate and severe groups (1.3931); it was regarded as simply a risk factor for every patient grouping.

Furthermore,[17] researchers looked at how 679 COVID-19-risk changes affected the process of gene expression in numerous kinds of immune cell systems. Expression of eleven genes that encode proteins was strongly associated with high COVID-19-risk variations, which are also associated with targeted gene promoters or cis-regulatory areas which interact with target promoters within the cell types, for instance, and found that the CCR2 activator in monocytes preferentially interacts with a functioning cis-regulatory location, which is probably the mechanism mediating the relationship between variations at the 3p21.31 risk locus and CCR2 expression in traditional monocytes. An additional investigator conducted a study to find variations in a single nucleotide in genes related to sensitivity or the severe effects of COVID-19.

In study, a cohort of 319 genetic DNA specimens collected from patients exhibiting different levels of disease severity, together with 78 controlling DNA, were analyzed. The investigation focused on the association between the odds of illness or life-threatening symptoms and the presence of seven single nucleotide polymorphisms. Statistical analysis revealed a significant correlation between the rs1799864 SNP located within the CCR2 gene and the likelihood of developing sickness or experiencing severe symptoms (allele A, OR = 2.21, P = 0.015).[18]

Conclusion

The study of immunological parameters indicated the level of CCR2 and genotyping were raised in individuals

who suffer from COVID-19. It is essential to remember that not all the alterations in biological processes could be exclusively referred to these elevated levels. Consequently, our research proposes further investigating additional cytokines stimulating inflammation and their corresponding receptors.

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Author contribution

All the authors listed have made a substantial, direct, and intellectual contribution to the work, and approved it for publication.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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