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Downregulation of CCL20 in Severe COVID-19 Infections and its Association with NLRP3 Gene Polymorphisms

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Abstract

Genetic factors are associated with the immunogenicity of coronavirus disease 2019 (COVID-19). This association reaches the level of cellular processes such as autophagy, ferroptosis, and pyroptosis, correlating with increased activity of the Nod-like receptor family, pyrin domain containing 3 (NLRP3) inflammasome. The present study focuses on exploring the linkage between severe COVID-19, CCL20 chemokines, and NLRP3 gene variants in an Iraqi sample. A case-control study enrolled 99 COVID-19 patients and 96 controls and measured CCL20 levels, disease severity, blood biomarkers, and NLRP3 SNPs (rs35829419, rs10754558, rs72771992, and rs10802501) using allele-specific primer methods. The results showed that the frequencies of NLRP3 *rs*35829419*A* allele (28.8 *vs*. 18.8 %; OR= 1.75; 95% CI = 1.09-2.81 ; $p < 0.013$) and genotype CA (45.5 vs 30.5 %; OR=1.82; 95% CI =1.01-3.26 ; $p < 0.032$) are related to susceptibility to COVID-19. The median value of CCL20 in severe COVID-19was 70.7 (range 63.4 – 81.6), being significantly lower than that in moderate cases (median 85.2; range 67.1 – 96.2). A negative correlation also existed between CCL20 and the levels of the inflammatory markers, such as ferritin, CRP, LDH, and D-dimer. The analysis of the relationship between the frequencies of NLRP3 SNPs alleles and genotypes with CCL20 level showed that the increases in the frequencies of rs10754558 C allele and CC genotype were correlated with the high median value of the CCL20 level compared with the low median value (48.8 vs. 33.3 %; OR=1.9; 95% CI = 1.24-2.91; $p < 0.004$ for the allele and 40.7 *vs*. 28.3%; OR= 2.05; 95% CI = 1.07-3.93; $p < 0.033$ for the genotype). Median levels of CCL20 stratified by SNP genotypes in the NLRP3 gene showed no significant relationship, except rs10802501 (81.58 *vs* 88.61 for AA genotype; 74.29 *vs* 81.58 for TT genotype; $p=0.045$). The study showed that the associations of several NLRP3 SNPs with altered CCL20 and other chemokines' levels can provide information about cellular inflammatory mechanisms that will be instrumental during making precise decisions for the treatment of disease.

Keywords: Iraqi population, SNPs, chemokine, Allele specific-primer method, CCL20,COVID-19

التنظيم المتدني لمستوىCCL20 مع الاصابات الحادة بالكوفيد 19 ومصاحبتها للتغايرات الجينية في NLRP3

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الخلاصة

العوامل الوراثية والمناعة تكون متصاحبة ومؤثرة في مرض كوفيد 19 بالاضافة الى انها تؤثر على مستوى العمليات الخلوية مثل autophagy, ferroptosis, and pyroptosis وهذا يتعلق مع زيادة نشاط NLRP3 وحدة المرض الفايروسي والتحورات المناعية

. ركزت الدراسة على توضيح العلاقة بين مرض الكوفيد 19 و CCL20 والتحورات الجينية في NLRP3 في العينات العراقية . تضمنت الدراسة 99 مريض و 96 سيطرة حيث تم تحديد نسبة CCL20 والعلامات البايلوجية الدموية والتغايرات في جين NLRP3 (SNP) (rs35829419, rs10754558, rs72771992, and rs10802501) بأستخدام طريقة allele-specific primer. أشارت النتائج الى ان الاليل A والنمط الجيني CA في rs35829419 NLRP3 له علاقة بالتحسس والاصابة بالكوفيد 19 (28.8 *vs.* 18.8 %; OR= 1.75; 95% CI = 1.09–2.81 ; $p < 0.013$ for allele), (45.5 vs 30.5 %; OR=1.82; 95% CI =1.01–3.26 ; $p < 0.032$ for genotype) مستوى CCL20 معنويا منخفض في الحالات الحادة من كوفيد 19 مقارنة بالحالات المعتدلة كذلك هناك علاقة سلبية بين CCL20 مع بعض العلامات الالتهابية مثل Ferritin, CRP, LDH, and D-dimer . عند دراسة العلاقة بين مستوى CCL20 مع التغايرات الجينية في مستقبلات NLRP3 وجد ان الاليل C والنمط الجيني CC يزداد مع المتوسط المرتفع CCL20 مقارنة مع المتوسط المنخفض, (48.8 *vs.* 33.3 %; OR=1.9; 95% CI = 1.24–2.91; $p < 0.004$ for allele), (40.7 *vs.* 28.3%; OR= 2.05; 95% CI = 1.07–3.93; $p < 0.033$ for genotype) .عند دراسة العلاقة بين مستوى CCL20 مع التغايرات الجينية في مستقبلات NLRP3 ظهر انه لا يوجد علاقة معنوية ماعدا في النمط الجيني AA rs10802501 (81.58 *vs* 88.61 for AA genotype,74.29 *vs* 81.58 for TT genotype,) p=0.045) .مستقبلا يمكن تسليط الضوء على انواع اخرى من Chemokines ودراسة دورها في مختلف الامراض

1. Introduction

In relation to COVID-19 infection caused by the SARS-CoV-2 virus, a bulk of research has been focusing on the roles of NLRP3 gene polymorphisms and CCL20 (also called MIP-3α), which functions as a co-regulator for autophagy, pyroptosis, and ferroptosis [1]. These processes play major roles in other diseases such as cancer and virus infections, including Covid-19. CCL20 is a pro-inflammatory chemokine that binds to CCR6, which is predominantly expressed by the Th17 cell subset, an important effector cell in anti-microbial defense and immune responses [2].This particular chemokine plays an important role in directing the immune cells to the area where infection has occurred to activate the immune system to combat the pathogens attacking the body [3]. The NLRP3 inflammasome is an important mediating factor in viral diseases such as SARS-CoV2 [4]. It involves the activation of interleukins in response to cell stress, infection, and pathogens' presence [5]. It often plays a role in the innate immunity which forms the first line of protection against various diseases [6]. Single nucleotide polymorphism (SNP) in NLRP3 may be associated with altered inflammation, which might affect the tendency to infection or severity of inflammatory responses [7]. The SNPs may lead to excessive activation of the inflammasome, resulting in cytokine storm (a state of excessive production of immune cells and their messengers) that have been observed in those with severe COVID-19)[8]. On the other hand, some SNPs may decrease inflammasome activity, which could result in reduced host defense mechanisms against viruses. Many studies have established the role of CCL20 in COVID-19 [9,10,11], but

only a few have addressed the relationship between NLRP3 gene SNP and CCL20 during COVID-19 infections. Analyzing this relationship may be useful in improving our knowledge about how genetic variances affect COVID-19 symptoms and immune responsiveness. It is important to recognize these people who are predisposed to severe illness to treat them and also develop interventions that may control their immune response.

2. Materials and Methods

2.1. Clinical specimens. We focused on a sample of 99 Iraqi COVID-19 patients attending Baghdad Teaching Hospital. The age range was 42 – 55 years for the patients and 37 – 51 years for the control group (n=96). The College of Sciences' Research Ethics Committee in the University of Baghdad, Iraq, approved the study protocol (Ref. No.: CSEC/0922/0084).The study involved two groups of patients; severe and moderate COVID-19 patients (n=31 and 68 respectively).WHO Interim Guidance defined the moderate case as that in patients infected with pneumonia,while the severe case involved pulse oxygen saturation (SpO2) $\leq$ 93% or rate of respiration $\geq$ 30 breaths/min, i.e.severe respiratory distress[12].The control subjects were without chronic diseases (diabetes and cardiovascular),without respiratory infections and negative for serum COVID-19 IgM and IgG antibodies tests.After 4–5 days of hospitalization,nasopharyngeal swabs were obtained from patients.The viral RNA was isolated by a Mini kit QIAamp Viral RNA. SARS was diagnosed by real-time polymerase chain reaction analysis using a commercial kit with the manufacturer's instructions being followed.Chest computerized tomography(CT)test confirmed the diagnosis.Venous blood (5 ml) was collected and spread into ethylene diamine tetra acetic acid (EDTA) and plain tubes (2 and 3 mL,respectively).To collect serum, the plain tube was left toclot,then centrifuged(4°Cfor15min).C-reactive protein(CRP) level was tested by using an electro-chemiluminescence immunoassay system. CCL20 level was detected in serum samples using the ELISA method, which was also adopted for the measurement of the levels of some inflammatory markers (ferritin, lactate dehydrogenase, and D-dimer). The STANDARD F D-dimer FIA kit is based on immunofluorescence technology.Ferritin level was evaluated by using miniVIDAS analyzer, which depends on fluorescent enzymatic detection based on the Enzyme Linked Fluorescent Assay (ELFA) (BioMerieux). An *in vitro* test was applied for the quantitative determination of LDH in the serum, based on an Electro-Chemiluminescence Immunoassay (Roche Cobas Integra 400 plus c systems, free from hemolysis).

2.2. Determination of polymorphisms of NLRP3 genes

To determine the roles of the variants in the genes of NLRP3, we used the allele-specific primer method to analyze the NLRP3 variants ofrs35829419, rs10754558, rs72771992, and rs10802501. EDTA blood was used to isolate genomic DNA with the gSYNC DNA extraction kit (Geneaid, Taiwan). To detect target SNPs, DNA was exposed to PCR. SNP data and DNA sequence of the NLRP3 gene were downloaded first (http://asia.ensembl. org), and then the relevant primers were designed by using the Amplifx program, as follows;

rs35829419 F C/A(CCGACACCTTGATATGGTGC)
rs35829419 R (TGCTCCAAGTAGCTTACAAGAAA)
rs72771992 F T/G(CCTCCATAGGGAAACCTTTCT)
rs72771992 R (AAAACTCAGCAAACAGAAAGAAAAA)
rs10802501 F T/A (CTCATATCATCATTCCCGCTAT)
rs10802501 R (TGACTTTTAAAATGCACCAAACA)
rs10754558FG/C(CAGCATCGGGTGTTGTTG)
rs10754558 R (CCAGCTACAAAAAGCATGGA)

The PCR was performed in a total volume of 25 µL, with 5 µL AccuPower PCR PreMix (Bioneer, Korea),3 µL DNA,1µL from each primer (forward and reverse),and 15µL deionized distilled water.The tube was transported to a thermal cycler(Eppendorf,Germany)that was programmed as follows:one cycle of denaturation (94˚C for 3 min), 35 denaturation cycles of (94 C for 30 s), annealing (51˚C for rs10754558 and rs10802501,53˚C for rs35829419, and 58 ˚C rs72771992 for 30 s),extension (72 ˚C for 30 s), and a final extension cycle (72 ˚C for 5 min). The PCR products were electrophoresed in agarose gel (1.5%; 5 V/cm^2 for 55 min) and a gel documentation system was used to visualize the migrating bands.

2.3. Statistical analysis

Percentages and numbers were depended on to define significant differences and categorical variables were evaluated by the two-tailed Fisher exact test. Continuous variables were tested for normality (Kolmogorov-Smirnov and Shapiro-Wilk test). Normally distributed variables were evaluated as mean values with standard deviation (SD), and the Student *t*-test was used to evaluate significant differences. The median and interquartilerange were used to express the nonparametric variables (skewed),and to conclude significant differences, the Mann-Whitney *U* test was used.Pearson's Chi-square test with an online calculator (https://www.had2know.org/academics/hardy-weinberg-equilibrium-calculator-2alleles.html) were used for the estimation of Hardy-Weinberg equilibrium (HWE) for the frequencies of SNPs. The odds ratio and its 95% confidence interval were used to evaluate the SNP and disease relationship. The software WinPepi version 11.65 was used to obtain these estimations. A probability (p) ≤ 0.05 was considered assignificant. Spearman's rank-order correlation was used. According to the median value of CCL20 in this analysis, patients and controls were divided into low and high production groups, with the high production group serving as the reference category. For statistical analysis, GraphPad Prism version 8.0.0 (San Diego, California, USA) and IBM SPSS Statistics 25.0 (Armonk, NY: IBM Corp.) were both utilized. The power of the sample size was calculated using the G*Power, version 3.1.9.2, software [a power analysis that compromise; error probability: 0.05; power (1- error probability): 0.80; effect sized: 0.55; actual power: 0.80].

3. Results

3.1 CCL20 levels in the sera of COVID-19 patients and the healthy group

Table 1 exhibits that the median levels of CCL20 in the patients who are >45 years old is lower than that in patients ≤ 45 years old [74.3 (range 63.4 – 84.3) vs. 85.2 (range 67.1 – 99.8); p = 0.026]. Additionally, there are no significant differences in the chemokine level between diabetes patients and non-diabetic participants, neither between males and females. The differences were significant between patients of severe and moderate groups [70.7 (63.4 – 81.6) vs. 85.2 (67.1 – 96.2); p = 0.0163].

Table 1: CCL20 levels in the sera of COVID-19 patients and the healthy group.

Characteristic		CCL20 median (IQR); pg/mL	
		Patients (no. 70)	**Control (no. 60)**
Age group	≤ 45	85.2 (67.1 – 99.8)	84.2 (71.4 – 92.1)
	> 45	74.3 (63.4 – 84.3)	80.4 (69.3 – 89.3)
	p-value	***p* = 0.026**	*p* = 0.468
Sex	Male	76.1 (63.4 – 96.1)	83.7 (71.1 – 90.5)
	Female	74.3 (64.3 – 85.2)	79.8 (70.1 – 88.9)
	p-value	*p* = 0.567	*p* = 0.683
Diabetes Mellites	Yes	74.3 (63.4 – 96.2)	**NA**
	No	78.1 (63.4 – 88.9)	
	p-value	*p* = 0.661	
Severity	Mild-moderate	85.2 (67.1 – 96.2)	**NA**
	Severe	70.7 (63.4 – 81.6)	
	p-value	***p* = 0.0163**	

IQR: Interquartile range; NA: Not applicable; *p*: Mann-Whitney *U* test probability.

3.2. CCL20 levels in the patients of COVID-19 and healthy subjects.

Figure 1 demonstrates that the median levels of CCL20 in the patients 79.58 (range 69.91 -99.87)] were lower than in the healthy group [82.75 (71.66-87.36); p = 0.351].

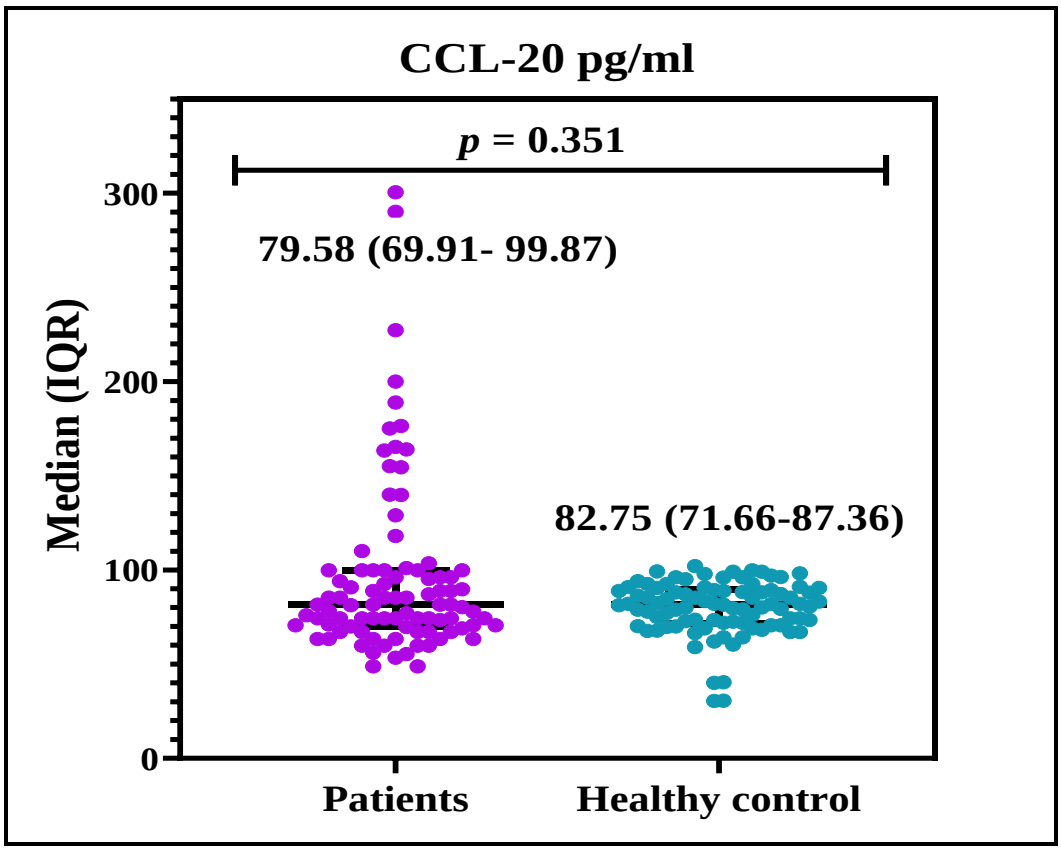

Figure 1: Scatter-dot plot of CCL20 levels in the patients of COVID-19 and healthy subjects.

3.3. Median levels of CCL20 in COVID-19 patients stratified by cycle number threshold (viral load)

Figure 2 shows appositive correlation between the median levels of CCL20 and CT value (viral load indicator) in COVID-19 patients.

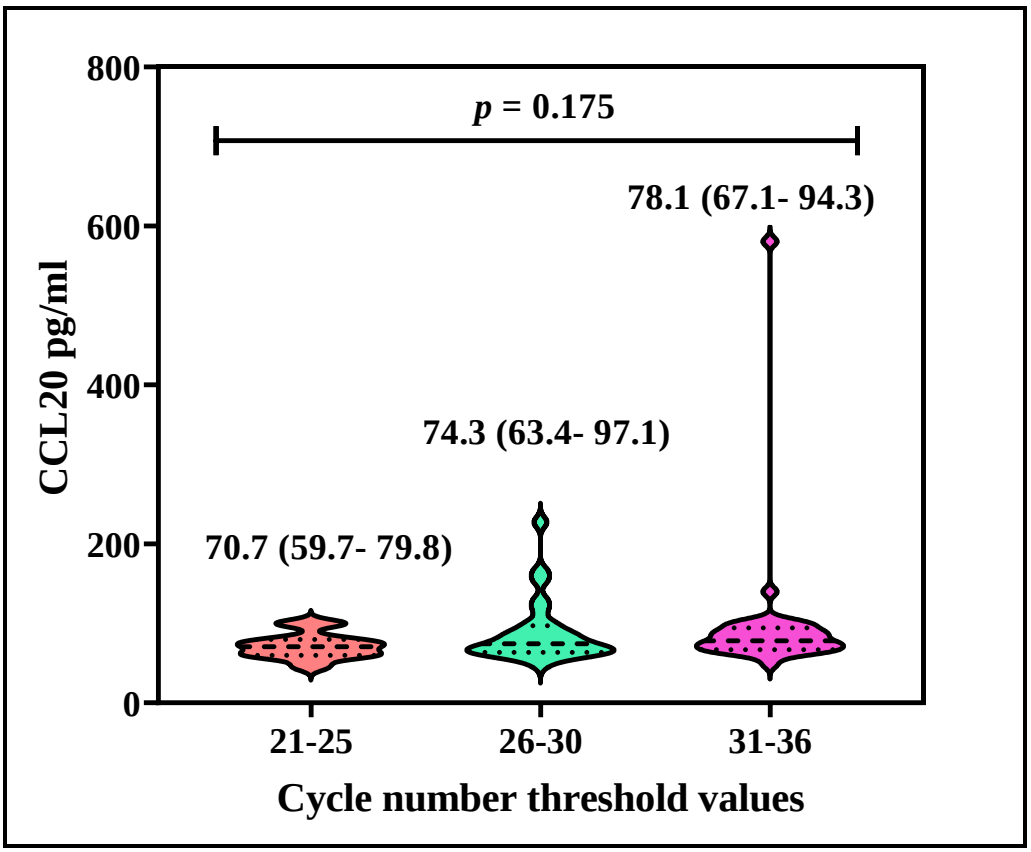

Figure 2: Median levels of CCL-20 in COVID-19 patients stratified by cycle number threshold (viral load); *p*: the probability of Kruskal-Wallis test between continuous variables.

3.4. Correlation between the levels of CCL20 and CRP, LDH, D-dimer, and Ferritin

There was a negative correlation between CCL20 levels and those of and ferritin, CRP, LDH**,** and D-dimer. According to Figure 3, the increase in ferritin, CRP, LDH**,** and D-dimer levels is associated with a drop in CCL20 levels (rs= -0.115, - 0.093, - 0.052, - 0.015, respectively).

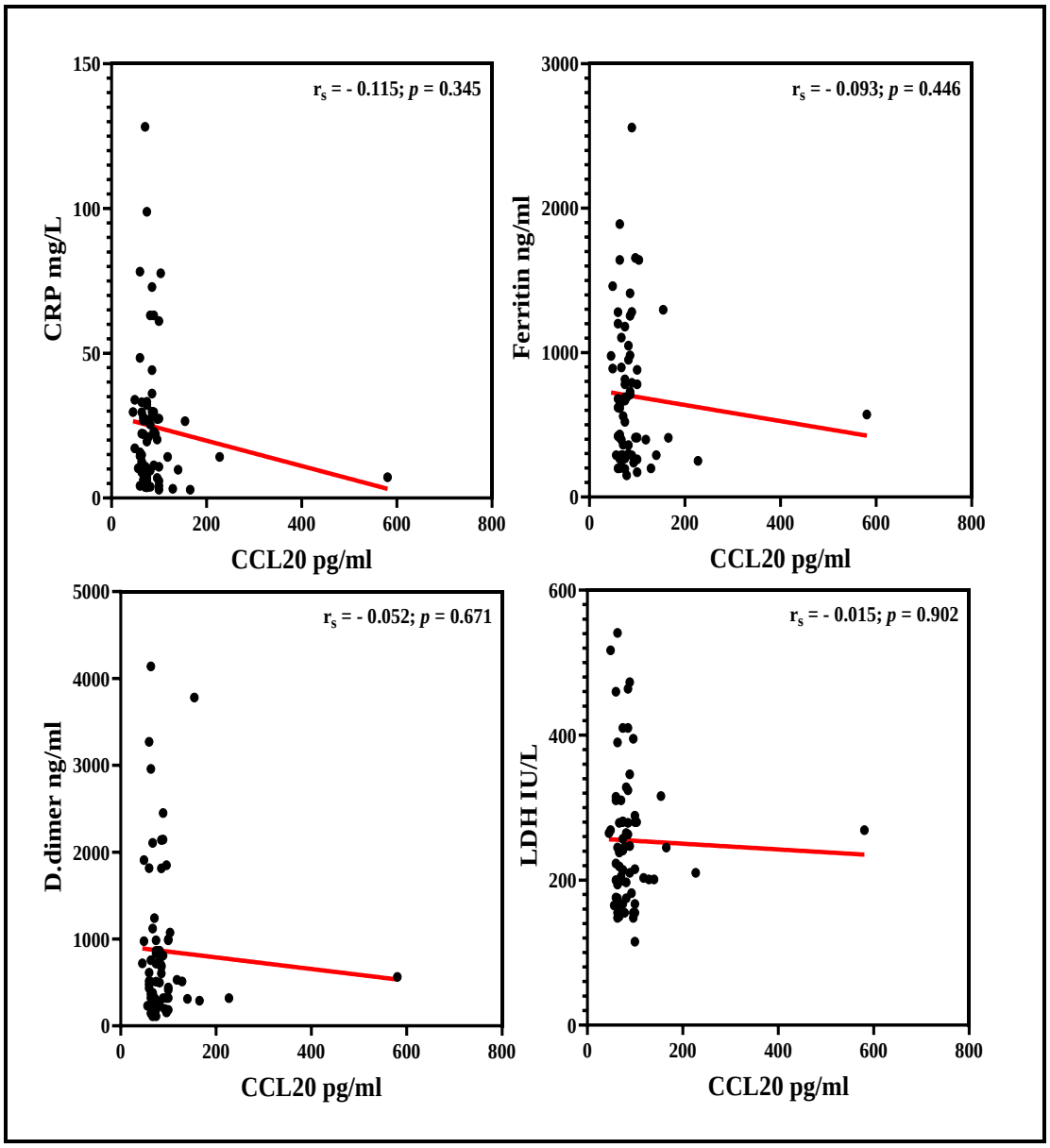

Figure 3 : Scatter plot of Spearman rank-order correlation coefficient (rs) analysis between CCL20 levels and those of CRP, ferritin, D.dimer, and LDH in COVID-19 patients.

3.5 *NLRP3* gene SNPs inCOVID-19 patients and healthy controls.

The genotype and allele frequencies did not display significant variations, except the rs35829419 allele and genotype CA which had higher frequencies in the patients than controls (28.8 *vs*. 18.8 %; OR= 1.75; 95% CI = 1.09-2.81 ; $p < 0.013$ for the allele and 45.5 vs 30.5 %; OR=1.82; 95% CI =1.01-3.26;p <0.032 for the genotype)(Table 2).

Table 2: Hardy-Weinberg and Logistic regression analyses of *NLRP3* gene SNPs in COVID-19 patients and healthy controls.

SNP	Allele/ genotype	COVID-19		HC		OR	95% CI	*p*-value(*pc*)
		N	%	N	%			
rs35829419	C	141	71.2	156	**81.3**	Reference		
C/A	A	57	28.8	36	18.8	1.75	1.09 – 2.81	**0.013**
	CC	48	48.5	62	**66**	Reference		
	CA	45	45.5	32	30.5	1.82	1.01 – 3.26	**0.032**
	AA	6	6.1	2	3.5	3.88	0.83 – 18.13	0.088
HWE-*p*-value		0.280		0.357				
rs10754558	G	149	75.3	130	67.7	Reference		
G/C	C	49	24.7	62	32.3	0.69	0.44 – 1.07	0.062
	GG	59	56.6	47	45.9	Reference		
	GC	31	37.3	36	43.7	0.69	0.37 – 1.26	0.147
	CC	9	6.1	13	10.4	0.55	0.22 – 1.38	0.152
HWE-*p*-value		0.113		0.163				
rs72771992	T	188	94.9	175	91.2	Reference		
T/G	G	10	5.1	17	8.9	0.55	0.24 – 1.23	0.164
	TT	89	89.9	81	84.4	Reference		
	TG	10	10.1	13	13.5	0.7	0.29 – 1.68	0.507
	GG	0	0	2	2.1	0.18	0.01 – 3.78	0.231
HWE-*p*-value		0.597		0.115				
rs10802501	T	147	74.2	130	67.7	Reference		
T/A	A	51	25.8	62	32.3	0.73	0.47 – 1.13	0.095
	TT	58	55.1	47	45.8	Reference		
	TA	31	38.3	36	43.7	0.7	0.38 – 1.29	0.161
	AA	10	6.6	13	10.5	0.62	0.26 – 1.52	0.214
HWE-*p*-value		0.071		0.163				

SNP (Single nucleotide polymorphism); HC(Healthy controls); HWE (Hardy-Weinberg equilibrium); CI (Confidence interval); OR (Odds ratio); *p*: Two-tailed Fisher's exact probability;The bold font refers to significant p-value.

3.6. NLRP3 Polymorphism

Figure 4 shows that conventional PCR was used to detect SNPs in the gene of NLRP3. The outcomes of gel electrophoresis exhibited three genotypes for each SNP; rs35829419 (CC,CA, AA), rs10754558 (GG,GC,CC) rs72771992 (TT,TG,GG), rs10802501 (TT, TA, AA). The sizes of PCR products of NLRP3 SNPs were as follows: 378 bp for rs35829419 (C/A),318 pb for rs10754558 (G/C), 458 pb for rs72771992 (T/G), and 305 pb forrs10802501 (T/A), as shown in Figure 4.

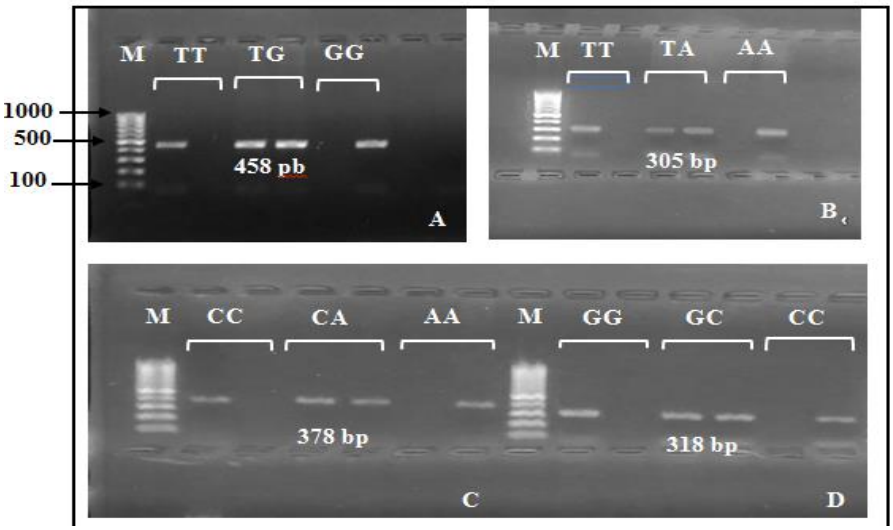

Figure 4: Representative images of agarose gel electrophoresis (1.5%; 5 V/cm2 for 55 minutes) of DNA–PCR products for *NLRP3* gene SNPs; **A**: 458 pb for rs72771992 (T/G), **B**: 305 bp for rs10802501 (T/A), **C**: 378 bp for rs35829419 (C/A), **D**: 318 bp for rs10754558 (G/C). M: DNA ladder (100bp).

3.7. Genotype and Allele frequencies of *NLRP*3 gene SNPs stratified by CCL20Table 3 shows genotype and allele frequencies of *NLRP*3 gene SNPs stratified by CCL20 level in COVID-19 patients. The results demonstrated no significant relationship between *NLRP*3 gene SNPs and CCL20 level, except **rs10754558** C allele and CC genotype which showed increased frequencies in high median values of CCL20 level compared with low median values (48.8 *vs*. 33.3 %; OR=1.9; 95% CI = 1.24-2.91; $p < 0.004$ for the allele and 40.7 *vs*. 28.3%; OR= 2.05; 95% CI = 1.07-3.93; $p < 0.033$ for the genotype

Table 3: Genotype and Allele frequencies of *NLRP*3 gene SNPs stratified by CCL20 level in COVID-19 patients and healthy control.

SNP	Allele/ genotype	CCL20				OR	95% CI	*p*-value
		High (> Median)		Median (≤ Low)				
		N	%	N	%			
rs35829419 C/A	C	158	84.1	133	77.3	Reference	0.38-1.10	0.110
	A	30	15.9	39	22.7	0.65		
	CC	67	70.6	51	59.8	Reference	0.31-1.12	
	CA	24	26.8	31	35.1	0.59	0.14-2.39	0.141
	AA	3	2.6	4	5.1	0.57		0.698
rs10754558 G/C	G	83	51.2	**132**	**66.7**	Reference		
	C	79	48.8	66	33.3	1.9	1.24-2.91	**0.004**
	GG	35	43.2	61	61.6	Reference		
	GC	13	16.1	10	10.1	2.27	0.92-5.61	0.099
	CC	33	**40.7**	28	28.3	2.05	1.07-3.93	**0.033**
rs72771992 T/G	T	152	88.4	177	94.2	Reference		
	G	20	11.6	11	5.8	2.12	0.99-4.51	0.06
	TT	69	80.2	83	88.3	Reference		
	TG	14	16.3	11	11.7	1.53	0.66-3.53	0.389
	GG	3	3.5	0	0	---	---	0.213
rs10802501 T/A	T	102	58.6	126	67.7	Reference		0.08
	A	72	41.4	60	32.3	1.48	0.96-2.28	
	TT	46	52.9	54	58.1	Reference		0.392
	TA	10	11.5	18	19.4	0.65	0.28-1.53	0.126
	AA	31	35.6	21	22.5	1.73	0.88-3.40	

SNP: Single nucleotide polymorphism; HWE: Hardy-Weinberg equilibrium; HC: Healthy controls; OR: Odds ratio; CI: Confidence interval; p: Two-tailed Fisher's exact probability; p: probability (significant *p*-value is indicated in bold).

3.8. Median levels of CCL-20 in COVID-19 patients and healthy control stratified by SNPs genotypes in *NLRP3*

Figure 5 shows the median levels of CCL20 in COVID-19 patients and healthy controls stratified by SNP genotypes in the NLRP3 gene. The results show no significant relationship between the NLRP3 gene SNPs and CCL20 level, except rs10802501 there is a significant difference in the median level of CCL20 between patient and control (81.58 vs 88.61 for AA genotype, 74.29 vs 81.58 for TT genotype) P 0.045

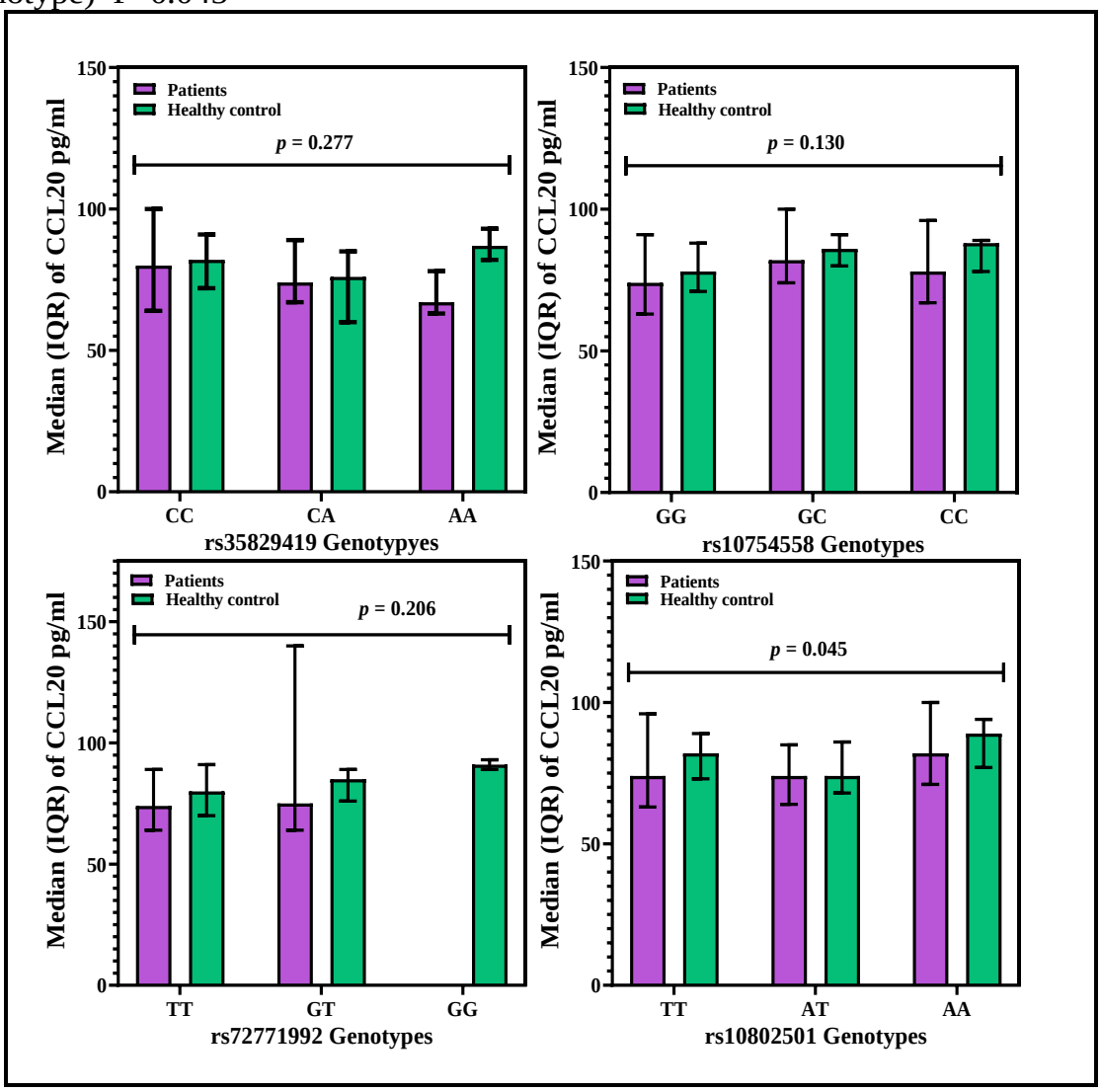

Figure 5: Median levels of CCL-20 in COVID-19 patients and healthy control stratified by SNPs genotypes in *NLRP3* gene; *p*: the probability of Kruskal test between continuous variables.

4. Discussion

This study was carried out to ascertain whether polymorphisms within the NLRP3 and CCL20 genes are capable of modulating the way COVID-19 affects an individual and the immunity displayed towards it. These findings are essential in identifying those people with higher chances of having a serious disease, providing informed treatment options, and ultimately can lead to designing targeted therapy to be more beneficial to the immune response.

Age was identified as a risk factor for CCL20 , whose level was significantly lower among elderly patients than their young counterparts. The immune system in older patients is weak because of numerous reasons, while there is a strong immune system in young patients. Numerous studies showed that aging leads to differences in adaptive and innate immunity. Immunosenescence leads to a decrease in the ability of immune systems to resist infections in older people [13,14]. However, sex and diabetes did not influence CCL20 levels. Moderate cases exhibited higher levels of CCL20 than severe ones, while those with Covid-19 had lower levels of CCL20 as compared to control subjects. Autophagy, pyroptosis, and ferroptosis were also part of the study. Analysis of bioinformatics exhibited the relationship between autophagy, programmed cell death, pyroptosis, ferroptosis, and CCL20 [15,16]. Autophagy is a conserved degradation of the cell that removes unnecessary or dysfunctional components through a lysosome-dependent regulated mechanism [17,18]. It permits the orderly degradation and recycling of cellular components, and also plays a main role in the homeostasis of non-starved cells [19].

Two manners of cell death are represented by pyroptosis and ferroptosis [20]. Many studies have recognized their anti-tumor action in many cancers [21]. Ferroptosis is a kind of programmed cell death related to iron and described by increased lipid peroxides [22]. It is biochemically and genetically distinct from apoptosis [23]. Inflammatory states from microbial or viral infections are induced by pyroptosis [24].

The study revealed that viral load was positively associated with CCL20 level, revealing its implication in suppressing viral growth. CCL20 was negatively correlated against markers for inflammation and tissue damage such as CRP, ferritin, LDH, and D-dimer. CRP concentration decreases when the inflammation or tissue damage is healed, rendering it a helpful indicator for tracking the severity of the disease [25].

Ferritin plays an essential role in cytokine storms by its pro-inflammatory and suppressive effects of the immune system. SARS CoV-2 causes inflammation and this leads to increased production of ferritin to reduce the iron effect [26,27]

LDH is an enzyme produced in all cells in the body and responsible for the production of energy/ LDH was used as an indicator for damage in tissues, being also related to interstitial lung disease and diseases of the liver. The value of D-dimers in COVID-19 patients was correlated to mortality and severe disease progression. Increased levels of D-dimers can refer to damage to the liver [28,29].The role of genetic variations in determining the susceptibility and severity of diseases, such as COVID 19, has been widely studied. There is one particular gene named NLRP3 that is responsible for triggering inflammatory cytokine activity as well as modulation of the immune system via an NLRP3 inflammasome [30]. The changes in this gene are associated with the function of the inflammasome [31].NLRP3 has been associated with various inflammatory diseases [32].

In our research, we examined how variations in the NLRP3 gene can relate to the severity of COVID-19. This study found that the NLPR3 rs 35829419 *A* allele and genotype CA are correlated to COVID-19 susceptibility. The polymorphism of NLRP3 rs35829419 C>A is found in 3'-UTR of the gene and may affect the expression and stability of NLRP3 mRNA. This variant is an increased function mutation that causes elevated secretion of IL-18 and IL-1β [33,34]. Therefore, NLRP3 rs35829419 affects a range of inflammatory diseases.

Our findings demonstrated a connection between NLRP3 gene variations and the increased risk of severe illness. Individuals who carried these variants were more likely to experience severe symptoms like respiratory distress, organ failure, and even mortality [35]. These results offer insights into understanding how genetics influence the severity of COVID-19 and suggest that genetic testing for NLRP3 polymorphisms could be used as a tool for predicting individuals at higher risk. In addition, we studied the correlation between variants in the NLRP3 gene and CCL20, a chemokine implicated in the recruitment of immune cells into inflamed sites [36,37]. In this case, different gene polymorphisms were related to varying degrees of CCL20 gene expression [38,39]. This implies that there is variable of the NLRP3 gene which alters immune reactions via the production of CCL20. Dysregulation of CCL20 expression has been implicated in several types of inflammation, including the proposed pathogenesis of COVID-19 [40,41].

Identification of genetic markers for disease severity and immune response has many implications in the clinic. It is also possible to use it to identify persons who are at high risk of suffering seriously. By such means, high-risk subjects can be identified for regular monitoring and instituted measures of mitigation of disease development and improved outcome. Additionally, knowing particular types of genetic variants related to serious diseases helps in planning treatment measures. It is possible to develop personalized medicine approaches, which will be based on the patient's genetic data. It may also enhance the effectiveness of therapy and minimize adverse responses. Additionally, it is important to understand how COVID-19 becomes severe due to hereditary aspects and the approach used to apply specialized interventions. Researchers have focused on the modulation of immune response to many chronic inflammations, including COVID-19 [42,43]. Therefore, directed actions may be designed aiming at the inhibition of NLRP3 inflammasome activation or stimulation of CCL20 secretion to enhance results among serious cases. Nevertheless, more studies should be conducted to explain how the NLRP3 genetic variations interact with CCL20 levels to render COVID-19 severe. However, it is crucial to acknowledge the limitations and inadequacies of such research. The sample size was relatively small and the study design was among a particular population from one region only. Therefore, more research should be conducted regarding the validity of the results across other populations.

The study further examined polymorphism of NLRP3 genes and CCL20 expression. However, there were other genes and immunological markers that might have contributed to COVID-19 severity [44,45]. Future studies will have to take a wide-ranging approach considering increased sample size and assessment of several genetic and immune markers. This study shows that there is a relationship between NLRP3 polymorphisms, CCL20 levels, and the extent of COVID-19. The frequencies of NLRP3rs10754558 C allele and CC genotype increase at high median levels of CCL20 . These results refer to the possible roles of the C allele and CC genotype in allowing the increase in the level of CCL20 that is necessary for the stimulation of immune response against COVID-19. The results point to the significance of differentiated genetics for disease outcome and immunity.This research is significant in terms of clinical implications such as identifying higher-risk people with serious illnesses, developing personalized therapies, and targeted intervention options. More research is needed to corroborate these findings, as well as to identify other genetic and immunologic markers potentially involved in COVID-19 severity.

Here, there is an association between a certain allele and genotype of the NLRP3 gene and susceptibility to COVID-19. NLRP3 gene is involved in the regulation of production of the inflammatory cytokines, with variations present in this gene leading to changes in its function [46]. It has been discovered that other components such as the inflammatory disorders and over-secretion of IL-1β are associated with the NLRP3 gene [47]. In particular, a polymorphism of a certain NLRP3 gene studied here is linked to numerous pathologies [48]. This is likely to alter the level at which the NLRP3 gene is expressed as well as elevate the secretion of IL-18 and IL-1β. Additionally, NLRP3 gene SNPs were linked with CCL20 levels, implying that particular alleles and genotypes raise the amount of CCL20 needed to fight COVID-19 immunological response.Expression ofCCL20 correlates with NLRP3 and has an association with inflammatory disease. It is used as a potential target of therapeutic approaches in several pathologies. The NLRP3 inflammasome must be stimulated to create mature IL-1β with the prospect of developing lung fibrosis [49]. Activated CCL20, in turn, is linked to inflammation and diseases caused by increased IL-1B secretion. Although there is still a lack of clear regulatory mechanisms behind CCL20's influence on NLRP3 activity, it involves transcriptional upregulation and ubiquitination [50]. CCL20 is critical in NLRP3 inflammasome activation that leads to IL-1β synthesis; this directly impacts immunity against COVID-19 illness. Analyzing NLRP3 gene polymorphisms and CCL20 concerning COVID-19 may be useful in improving our knowledge about how genetic variance affects COVID-19 symptoms and immune responsiveness development. It is important to recognize people who are predisposed to severe illness to treat them and also develop interventions that may control their immune response,As well as, understanding the characteristics of the SARS-CoV-2 genome and developing systems to monitor SARS-CoV-2 during the pandemic are critical steps for controlling this disease [51]. The study contains some limitations.First, the connections between parameters was not clear because of the small sample size of the patients and controls. Second, the lack of information sources about the relationship between CCL20 and NLRP3 in CoVID-19. Also the relationship between CCL20 and other cytokine was no investigated in this context.

Conclusions

The low level of CCL20 in patients with severe COVID infection and its inverse relationship with systemic inflammation markers suggest that the chemokine is involved in the immunologic response to SARS-CoV-2 and may serve as an index of illness deterioration.

A thorough analysis of CCL20 and NLPR3 gene polymorphism should serve as an inspiration for future studies related to COVID-19 as well as inflammation diseases in general. This opportunity could be utilized in clinical practice in the future to develop better diagnostics tests, including tailor-made therapies targeted at minimizing the seriousness of COVID-19.

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Conflict of interest

The authors declare that there were no conflicts of interest.

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