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Relation of CXCL10 and CXCR3 Gene Polymorphisms with COVID-19 Severity and its Serum Biochemical Markers

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Abstract

CXCL10 (rs201830102) is a chemokine involved in immune cell recruitment, while its receptor, CXCR3 (rs779120264), mediates immune responses through the activation of T cells. These genes are critical in the immune response to viral infections, including COVID-19. The study aimed to explore the relationship between polymorphisms in the CXCL10 gene and CXCR3 receptor with disease severity in COVID-19 patients. In this cross-sectional analytical study, 100 COVID-19 patients were enrolled after ethical approval, and written informed consent was obtained from each participant. Polymorphisms in CXCL10 and CXCR3 were analyzed by sequencing, while biochemical and hematological parameters were assessed using appropriate methods. Descriptive and inferential statistics were employed to analyze continuous and categorical data. Significant associations were observed between severe COVID-19 cases and elevated levels of serum D-dimer, ferritin, random blood sugar (RBS), neutrophils, and erythrocyte sedimentation rate (ESR), along with reduced hemoglobin levels. Lymphocyte and platelet counts were significantly lower with increased disease severity. The wild genotype of CXCL10 was notably associated with elevated ferritin levels, suggesting that certain gene variants may offer protective effects. However, no significant correlation was found between CXCR3 and CXCL10 polymorphisms and other serum biomarkers. Our study confirmed a significant rise in serum D-dimer, ferritin, RBS, neutrophils, and ESR, along with a reduction in hemoglobin, lymphocyte, and platelet counts in severe COVID-19 cases compared to mild ones. Notably, mutations in the CXCL10 gene were linked to less severe COVID-19 outcomes.

Keywords: COVID-19, CXCL10 Gene, CXCR3 Receptor, Single Nucleotide Polymorphism

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INTRODUCTION

The viral pandemic called the coronavirus disease 2019 (COVID-19) started in East Asia had rapidly spread to the rest of the world.[1] One of the most clinically significant effects of COVID-19 infection in such patients was acute respiratory distress syndrome (ARDS). The most frequently occurring consequence in COVID-19 infection patients was initially pneumonia. However, ARDS, the distinguishing feature of immune-mediated clinical consequence gradually follows COVID-19-related pneumonia.[2]

ARDS may be defined as an uncontrolled and generalized inflammatory response, potentially responsible for a significant number of deaths among infected patients. ARDS can occur in a variety of clinical situations including pneumonia, sepsis, pancreatitis, blood transfusion being associated with an elevated mortality. Cytokine storm occurs during infection of several viruses including influenza viruses and coronaviruses,[3,4] and is the main reason for acute lung injury and subsequent development of ARDS. In COVID-19 infection, ARDS represents the most serious consequence of the so-called "cytokine storm".[5,6]

An important factor in the surge of cytokines associated with COVID-19 and the onset of the profound lung dysfunction seen in these individuals is the pro-inflammatory chemokine CXCL10.[7] It is also known that this chemokine contributes to nerve degeneration brought on by coronaviruses. Chemokines are a subgroup of cytokines and have low molecular weight. They have the peculiar property of being able to chemo-attract cells called leukocyte and other antibody-producing cells to the location of injury or infection, which helps to trigger an inflammatory reaction.[8,9]

The CXCL10 gene, located on chromosome 4 at band q21, encodes a protein with a molecular weight of approximately 12 kDa. This protein contains two internal disulfide bonds, which are essential for stabilizing its structure. After cleavage, a 10 kDa form of the protein, containing four conserved cysteine residues in the N-terminal region, is produced and released. These cysteines are important for forming disulfide bridges that help maintain the protein's characteristic structure, known as the chemokine fold, which consists of a three-stranded beta sheet overlaid by an alpha helix. Unlike some other CXC chemokines, CXCL10 lacks the tripeptide motif "Glu-Leu-Arg", which is associated with anti-angiogenic properties. However, CXCL10 plays a key role in immune response regulation, particularly through its interaction with receptors such as CXCR3. Specific regions of the protein, particularly in the loops between its structural elements, are involved in receptor binding, and these loops are stabilized by the disulfide bonds. Through these interactions, CXCL10 helps recruit immune cells, particularly T cells, to sites of inflammation or infection. Several extrinsic cues, like IFN- and bacterial substances like lipopolysaccharides, control the production of CXCL10.[10] The chemokine CXCL10, also known as IP-10 or interferon-inducible 10 kDa protein, plays a crucial part in the onset and regulation of immune reactions. The interferon-gamma is required to produce CXCL10 by cluster immune cells. CXCL10 is a Th1-chemokine that can draw other Th1 lymphocytes. It expresses itself via the seven transmembrane G-protein coupled receptor CXCR3. Immune cells move towards the infection location as a result of the chemokine CXCL10 binding to its receptor CXCR3. Therefore, the emergence of various disorders is linked to the hyperactivity of the CXCL10-CXCR3 axis.[11] Until the adaptive immune system is activated, this chemokine is known to promote viral eradication as part of the host anti-viral response. It is surplus in circulation in respiratory tract infections.[12]

Studies that looked at the prospect of finding biomarkers for the severity and progression of COVID-19 revealed that CXCL10 cytokines were markedly higher in cases when compared to healthy controls.[13,14] It has thus been postulated that the CXCL10-CXCR3 axis may be involved in the onset of COVID-19 and the level of the disease severity may be related to cytokines and chemokines SNPs. CXCL10 gene polymorphism and the degree of severity of SARS-CoV-2 illness is therefore justified. This made us to undertake this study to study the pattern of CXCL10 and CXCL3 gene polymorphisms and its association with biochemical and hematological parameters and disease outcome.

The objectives of the study were to determine the pattern of single nucleotide polymorphism of CXCL10 gene (rs201830102) and CXCR3 gene (rs779120264) in patients with COVID-19 and to determine the association of biochemical parameters and its expression with CXCL10/CXCR3 gene and the disease outcome.

MATERIALS AND METHODS

In this cross-sectional study, one hundred patients diagnosed of COVID-19 aged more than 18 years were participated. The diagnosis and severity of the disease was staged according to comprehensive guidelines for management of COVID-19 patients published by Director General of Health Services, Ministry of Health and Family Welfare (MoHF), Government of India. This study was conducted in the Department of Biochemistry, in collaboration with COVID-19 task force of Justice KS Hegde Charitable Hospital and Molecular Genetics Division of Central research laboratory/Virology of KS Hegde Medical Academy, Mangalore, India. The study was conducted after the approval of Central Ethics Committee, Nitte (Deemed to be University) (INST.EC/EC/110/2021-22). Informed consent was also obtained from the study subjects.

Patients who were clinically diagnosed of hypertension, pre-existing co-morbid conditions were excluded from the study.

The demographic (age, gender), clinical and laboratory parameters such as medical histories, clinical symptoms (fever, cough, expectoration, dyspnea, chest pain, diarrhea, headache, anorexia, nausea and vomiting), vital signs (temperature, heart rate, respiratory rate, oxygen saturation, blood pressure) were collected from the patient medical records and recorded in the data collection form.

Two milliliters of venous fasting blood sample was collected with the standard precautions from COVID-19 diagnosed patients from COVID wards/ ICU in EDTA, plain, and fluoride tubes after wearing personal protective equipment. The blood samples were centrifuged at 3000 rpm for 15 minutes. EDTA sample was used for genotyping, plain blood for serum insulin whereas fluoride sample was analyzed for fasting blood glucose estimation. D-dimer, ferritin and other biochemical and hematological parameters were taken from the medical records

DNA was extracted and genotyped using EDTA blood. Miller's DNA isolation technique was modified, and the DNA was isolated and stored at -20°C. The DNA was amplified by PCR and pattern was analyzed by Sanger sequencing technique (Figure) to study the SNP for CXCL10 (rs201830102) and CXCR3 (rs779120264). The primers were designed by using the software Primerblast. The suitable forward and reverse primers.
CXCL10: (rs201830102)
Forward Primer: 5'- TGTGATCCATTCCTCCTCAGAT - 3'
Reverse Primer: 5'-ACAACCAGGGAAGTGATAATCAGA- 3'
CXCR3: (rs779120264)
Forward Primer: 5'-CCACCTAGCTGTAGCAGACA-3'
Reverse Primer: 5'-TCCCGGGAGCGAGGATATT-3'

By electrophoresis on 0.8% agarose gel with ethidium bromide (0.5 g/mL) in TAE buffer, the DNA's quality was examined. The quantification and purity of DNA was checked by the spectrophotometer (ratio of OD260/OD280) after the amplification of DNA, that is after PCR. DNA concentration was calculated using the following formula:

Concentration (µg/mL) of DNA in original solution= Absorbance x 100 x 50 µg/mL.

Fasting blood sugar, urea, creatinine, total protein, albumin, globulin, electrolytes, D dimer, ferritin, SGOT, SGPT were estimated by commercially available reagent kits using fully automated clinical chemistry analyzer, Cobas C-311. Fasting insulin was assayed by ELISA. Hb %, neutrophil %, lymphocyte %, platelet count and total leucocyte count was done using fully automated hematology instrument Sysmex XN 1000.

Descriptive statistics of the exploratory and outcome variables was calculated be mean ± SD, percentage, median ± IQR based on normality of the data. Hardy Weinberg equilibrium was performed in the cases for each genotype variant. Chi square analysis was used to find the association of gene polymorphisms with severity of infection and serum biochemical parameters. Metabolic parameters were compared between different stages of severity (severe, moderate and mild) by Kruskal-Wallis test and one-way ANOVA test.

Table 1. Hardy Weinberg Equilibrium of CXCL10 genotype in study participants

CXCL10 (rs201830102) C/T	Cases		Chi square value	P value
	Expected	Observed		
Dominant homozygotes (CC)	97.02	97		
Heterozygotes (CT)	2.95	03	0.0232	0.985
Recessive Homozygotes (TT)	0.025	0		

Table 2. Hardy Weinberg Equilibrium of CXCR3 genotype in study participants

CXCR3 (rs779120264)	Cases		Chi square value	P value
	Expected	Observed		
Dominant homozygotes (AA)	86.49	92		
Heterozygotes (AC)	13.02	02	71.6376	0
Recessive Homozygotes (CC)	0.49	06		

Dunn's multiple comparison test was used to determine the statistical differences between the biochemical parameters in the three groups.

RESULTS

The distribution of genotypes and alleles of CXCL10 (rs201830102) and CXCR3 (rs779120264) gene variants are provided in Table 1 and Table 2. The chromatogram of the sequenced genes are represented in Figure.

The genotype frequency distributions for CXCL10 (rs201830102) showed no significant deviation from HWE in COVID-19 cases, suggesting that the alleles were in equilibrium and CXCR3 (rs779120264) gene variant deviated significantly from HWE in COVID-19 cases (P < 0.05), suggesting that alleles were not in equilibrium. The percentage of mutations in the CXCL10 gene (rs201830102) is very low, with 3% of patients showing the heterozygous genotype (CT) and no patients showing the recessive homozygous genotype (TT). In contrast, the CXCR3 gene (rs779120264) showed 2% of patients with the heterozygous genotype (AC) and 6% with the recessive homozygous genotype (CC), which gives a combined prevalence of 8% for patients carrying either the heterozygous or recessive genotype. (Table 1 and Table 2) CXCL10 showed no deviation from Hardy-Weinberg equilibrium (p = 0.985), while CXCR3 significantly deviated (p <

0.05). These results emphasize the importance of CXCR3 gene variants in the patient cohort studied.

Random blood sugar (RBS) level varied widely among severe, moderate, mild case and this variation was statistically significant (P < 0.0001) (Table 3). Post hoc test showed highly significant values between RBS values of severe and mild cases (P < 0.001) and significant value (P < 0.05) between moderate and mild cases. Blood urea also showed highly significant difference in various severity of COVID-19 infection (P < 0.0001) (Table 3). AST and AST levels in moderate and severe were significantly high compared to mild cases of COVID-19 cases, respectively (P-0.0003) ALT (P-0.0046) (Table 4). Total protein (P-0.009) and serum albumin levels (P < 0.0001) were significantly less in moderate and severe cases of COVID-19 cases as shown in Table 4.

There was a significant difference in all hematological parameters except total leucocyte count in different grades of COVID-19 severity. Hemoglobin (P = 0.0156), lymphocyte (P = 0.0049 and platelet counts (P < 0.013) were significantly less in moderate and severe cases compared to mild cases while rest of the parameters such as neutrophils (P = 0.0016), ESR (P < 0.0001), Ferritin (P < 0.0001) and D-dimer (P < 0.0001) showed highly significant difference in their values. All these parameters were higher in moderate and severe cases of COVID-19 compared to mild cases (Table 5).

There was no association of gene polymorphism with D dimer, SGOT, ESR, RBS, HB, lymphocytes and disease severity (P > 0.05) (Tables 6-11). Increased ferritin levels showed significant association between wild (CC) and mutant type of CXCL10 gene polymorphism (P=0.0123) (Table 12), as was neutrophil count in moderate COVID-19 infection (P=0.016) (Table 13). Additionally, increased platelet count in mild cases showed highly significant association between wild (CC) and mutant variant of CXCL10 gene polymorphism (P = 0.0003) (Table 14).

There were no significant associations found between CXCR3 gene polymorphisms and the biochemical or hematological parameters measured (D-dimer, ferritin, neutrophils, SGOT, ESR, RBS, and hemoglobin) in severe, moderate, or mild COVID-19 cases (Tables 15-21). This suggests that, unlike CXCL10, the CXCR3 gene polymorphisms do not significantly influence these clinical markers in relation to COVID-19 severity.

DISCUSSION

Ichikawa and colleagues elegantly demonstrated that the CXCL10-CXCR3 binding signalling plays a major role in the exacerbation of ARDS in mice.[15] ARDS was induced by viral infection (with influenza H5N1 virus) in both wild-type and CXCL10/CXCR3 knock-out mice. The authors observed that wild type mice developing ARDS showed a concomitant increase of CXCL10 levels mainly due to an increased secretion by infiltrating neutrophils, which induced an autocrine loop mechanism on the chemotaxis of inflamed neutrophils, leading to fulminant pulmonary inflammation. On the other hand, CXCL10 or CXCR3 knock-out mice showed less severe lung injury.[16,17] Additionally, in ARDS patients undergoing mechanical ventilation, CXCL10 and CXCR3 levels were measured, revealing a significant correlation between elevated CXCL10 and CXCR3 expression and the severity of ventilator-induced lung injury, which appears to be mediated by mast cell chemotaxis.[18]

In our study, increased ferritin levels, an inflammatory marker, were significantly associated with the CXCL10 gene polymorphism, specifically the wild-type (CC) genotype, in severe COVID-19 cases. Additionally, the same genotype (CC) showed a significant decrease in platelet count in severe cases. Moreover, CXCR3 gene polymorphisms
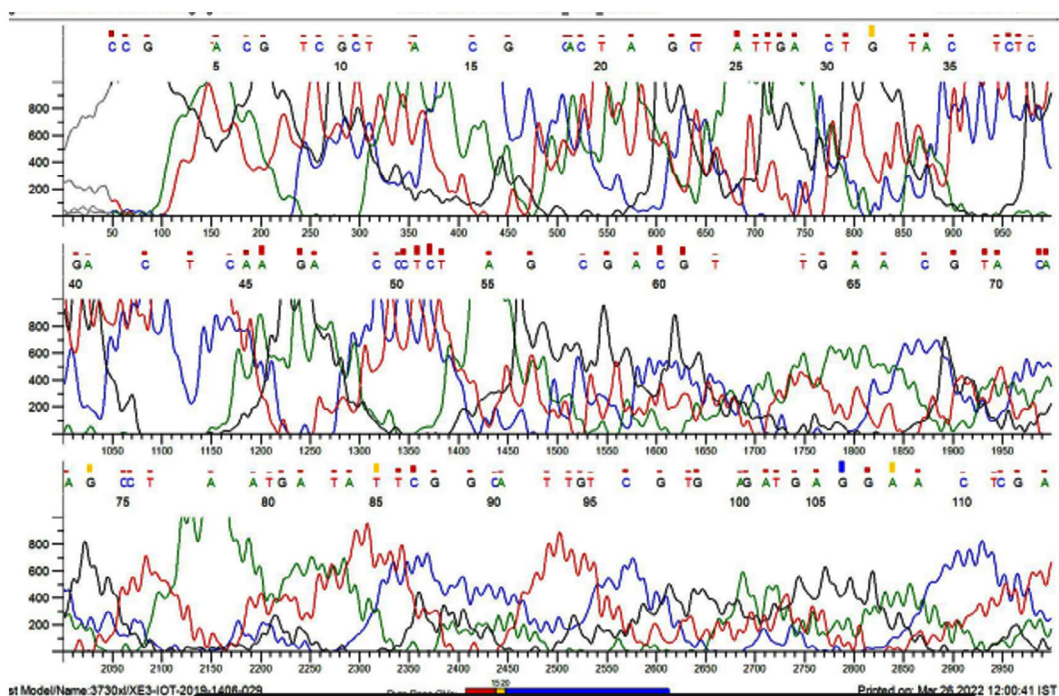

Figure. Chromatogram of sequenced genes

were observed in a significant number of patients, although no significant associations were found between CXCR3 polymorphisms and biochemical or hematological markers. In a study published in 2020, Chua et al. examined the immune response and pathways linked to severe COVID-19. The authors stated that when compared to the moderate cases, the expression of the various chemokine-encoding genes CCL2, CCL3, CCL20, CXCL1, CXCL3, and CXCL10 increased noticeably

Table 3. Comparison of biochemical parameters in various severity COVID-19 groups

Parameters	Mild	Moderate	Severe	P value
FBS (mg/dL)	142 (80-224)	146 (127-170)	141.5 (73-196)	0.5988
Insulin (µIU/mL)	2.77 (0.020-34.8)	6.37 (1.71-32.5)	2.67 (0.02-56.716)	0.1588
RBS (mg/dL)	105 (77-292)	150 (93-450)	169 (90-497)	<0.0001**
C-peptide (ng/mL)	1.7 (0.27-7.75)	2 (1.25-4.9)	1.89 (0.06-7.75)	0.28
Leptin (ng/mL)	60.92 (2.7-92.88)	69.23 (30.5-98.03)	56.295 (4.34-102.6)	0.73
Potassium (mEq/dL)	4.081 ± 0.4480	4.148 ± 0.616	4.161 ± 0.6190	0.8122
Uric acid (mg/dL)	4.94 (1.59-10.41)	3.43 (1.93-5.59)	3.98 (1.7-10.62)	0.1805
Sodium (mEq/dL)	138 (124-144)	135 (129-140)	137 (116-148)	0.0607
Creatinine (mg/dL)	0.86 (0.48-3.8)	0.725 (0.53-1.24)	0.85 (0.44-8.72)	0.429
Urea (mg/mL)	19.3 (10.4-82.30)	21.8 (16.9-30.3)	34.1 (12.3-123.5)	<0.001**

P < 0.0001, RBS between mild and severe COVID cases
P < 0.001, Blood urea level between mild and severe cases

Table 4. Comparison of hepatic parameters in various severity COVID-19 groups

Parameters	Mild	Moderate	Severe	P value
Albumin (g/dL)	4.29 ± 0.88	3.89 ± 0.377	3.54 ± 0.44	<0.0001**
Globulin (g/dL)	2.95 (1.98-4.61)	3.055 (2.41-3.98)	2.925 (2.17-4.43)	0.49
TP (g/dL)	7.3 (4.1-8.1)	7.15 (6.1-8.0)	6.5 (5-8.6)	0.0009**
ALP (IU/L)	80 (38-248)	77 (40-205)	85 (34-229)	0.872
AST (IU/L)	24.8 (14.1- 143.7)	44.7 (11-187.7)	52.5 (9.5-452.7)	0.0003**
ALT (IU/L)	23.20 (7.2-85.10)	42.15 (13.3-124)	32.5 (6.4-877.2)	0.0046**

P < 0.0001 in serum albumin, P < 0.0005 in AST, P < 0.005 in ALT and P < 0.001 in total protein values between mild and severe cases

Table 5. Comparison of haematological parameters in various severity COVID-19 groups

Parameters	Mild	Moderate	Severe	P value
Haemoglobin (g/dl)	13 (1.8-17)	13.1 (9.6-14.1)	11.6 (3.1-15.2)	0.0156*
ESR mm/hr	19.50 (2-74)	53 (30-98)	56 (2-100)	<0.0001**
TLC (cmm)	7814.28 ± 3139.3	8370 ± 4323.6	8327.9 ± 3287.5	0.7753
Neutrophils (cmm)	79.5 (50-93)	81 (70-96)	89 (36-96)	0.0016**
Lymphocyte (cmm)	19 (5-48)	18 (2-29)	10 (5-48)	0.0049**
Platelet count (Lakh/mm^3)	240000 (4000-468000)	232000 (120000-493000)	182000 (28000-514000)	<0.0130*
Ferritin (µg/mL)	135.15 (10.41-1542)	464 (126.7-1064)	643.85 (5.82-4283)	<0.0001**
D-dimer (µg/mL)	0.24 (0.01-20.66)	0.45 (0.12-4.51)	1.305 (0.01-24.56)	<0.0001**

P < 0.0001 in ESR, Ferritin, D-dimer values between mild and severe cases
P < 0.005 in Neutrophils (mild), Lymphocytes (mild, moderate), with severe cases
P < 0.05 in haemoglobin between mild and severe cases
P < 0.05 in platelet count between mild, moderate with severe cases

in the critical cases, which may have boosted the recruitment of cells from the immune system to the areas of inflammation (lung), leading to respiratory distress.[19]

Role of hepatic markers with severity of COVID-19 infections

Patients with severe COVID symptoms in our study had significantly higher AST and ALT levels which was similar with a study done with

Table 6. Association of expression of D-dimer with CXCL10 gene polymorphism in various stages of COVID-19

Severity of COVID	D-Dimer µg/mL	CC	CT+TT	Chi Square value	P VALUE	OR (95% CI)
Severe	<0.5 µg/mL	09	00	0.255	0.613	0 (0 to 0.28)
	>0.5 µg/mL	35	01			
Moderate	<0.5 µg/mL	06	00	P value could not be calculated as dataset is 0		
	>0.5 µg/mL	05	00			
Mild	<0.5 µg/mL	23	00	0.2524	0 (0 to 1.420)	0.2524
	>0.5 µg/mL	17	01			

Table 7. Association of SGOT levels with CXCL10 gene polymorphism in various stages of COVID-19

Severity of COVID	SGOT	CC	CT+TT	Chi Square value	P VALUE	OR (95% CI)
Severe	<59	24	01	0.03629	0.9520	1.091
	>59	22	01			(0.05515 to 21.50)
Moderate	<59	06	00	P value could not be calculated as one of the data set was 0		
	>59	05	00			
Mild	<59	30	01	0.3306	0.5653	0
	>59	10	00			(0 to 27.90)

Table 8. Association of ESR levels with CXCL10 gene polymorphism in various stages of COVID-19

Severity of COVID	ESR	CC	CT+TT	Chi Square value	P VALUE	OR (95% CI)
Severe	<20	04	01	3.504	0.0612	0.09524
	>20	42	01			(0.004951 to 2.237)
Moderate	<20	00	00	P value could not be calculated as one of the data set was 0		
	>20	11	00			
Mild	<20	17	00	0.7260	0.3942	0
	>20	23	01			(0 to 0.07870)

Table 9. Association of RBS levels with CXCL10 gene polymorphism in various stages of COVID-19

Severity of COVID	RBS	CC	CT+TT	Chi Square value	P VALUE	OR (95% CI)
Severe	<140 mg/dL	15	01	0.2609	0.6095	0.4839
	>140 mg/dL	31	01			(0.02479 to 9.761)
Moderate	<140 mg/dL	03	00	P value could not be calculated as sample size was 0		
	>140 mg/dL	08	00			
Mild	<140 mg/dL	24	01	0.6560	0.4180	0
	>140 mg/dL	16	00			(0 to 14.06)

increased AST and ALT levels in COVID cases by Hwaiz et al.[20] It was also said that a very high level of AST could predict overall survival in individuals with severe COVID-19. Similarly, our study exhibited significant association of serum

AST with disease severity. AST levels were elevated more than the normal range in patients with severe symptoms, whereas in mild and moderate these levels were within the normal range. The hepatic damage could be due to consumption of

Table 10. Association of Hb% levels with CXCL10 gene polymorphism in various stages of COVID-19

Severity of COVID	Hb%	CC	CT+TT	Chi Square value	P VALUE	OR (95% CI)
Severe	<15	41	02	0.2427	0.6223	0
	>15	05	00			(0 to 19.72)
Moderate	<15	10	01	P value could not be calculated as		
	>15	00	00	sample size was 0		
Mild	<15	25	01	0.5913	0.4419	0
	>15	15	00			(0 to 15.60)

Table 11. Association of lymphocyte levels with CXCL10 gene polymorphism in various stages of COVID-19

Severity of COVID	Lymphocytes	CC	CT+TT	Chi Square value	P VALUE	OR (95% CI)
Severe	<20	32	02	0.8593	0.3539	0
	>20	14	00			(0 to 5.279)
Moderate	<20	06	00			
	>20	05	00			
Mild	<20	20	01	0.9762	0.3231	0
						(0 to 9.450)

Table 12. Association of ferritin levels with CXCL10 gene polymorphism in various stages of COVID-19

Severity of COVID	Serum Ferritin	CC	CT+TT	Chi Square value	P VALUE	OR (95% CI)
Severe	<464 ng/mL	12	02	6.26	0.0123*	0
	>464 ng/mL	34	00			(0 to 0.6869)
Moderate	<464 ng/mL	03	03	0.110	0.7401	0.6667
	>464 ng/mL	03	02			(0.07613 to 5.848)
Mild	<464 ng/mL	27	01	0.4511	0.5018	0
	>464 ng/mL	13	00			(0 to 21.00)

Table 13. Association of neutrophil count with CXCL10 gene polymorphism in various stages of COVID-19

Severity of COVID	Neutrophils	CC	CT+TT	Chi Square value	P VALUE	OR (95% CI)
Severe	<80%	08	00	0.4174	0.5182	0
	>80%	38	02			(0 to 0.00899)
Moderate	<80%	05	07	5.727	0.0167*	0
	>80%	06	00			(0 to 0.6999)
Mild	<80%	19	00	0.8852	0.3468	0
	>80%	21	01			(0 to 0.09596)

antipyretic drugs and antiviral medications, oxygen deficiency and ATP consumption in liver cells that can release ROS and trigger pro-inflammatory factors resulting in hepatocyte damage.[21]

Role of hematological markers with severity of COVID-19 infections

In the current study, COVID-19 patients had a drop in lymphocyte that substantially correlated with the severe COVID cases. These

Table 14. Association of platelet levels with CXCL10 gene polymorphism in various stages of COVID-19

Severity of COVID	Platelets	CC	CT+TT	Chi Square value	P VALUE	OR (95% CI)
Severe	<1,50,000	11	01	0.6957	0.4042	0.3143
	>1,50,000	35	01			(0.01617 to 6.479)
Moderate	<1,50,000	01	00			
	>1,50,000	11	00			
Mild	<1,50,000	02	01	12.98	0.0003**	0
	>1,50,000	38	00			(0 to 0.7105)

Table 15. Association of D-dimer levels with CXCR3 (rs779120264) gene polymorphism and COVID-19 cases

Severity of COVID	D-Dimer µg/mL	AA	AC+CC	Chi Square value	P VALUE	OR (95% CI)
Severe	<0.5µg/mL	09	00	0.4816	0.487	0
	>0.5µg/mL	37	02			(0 to 0.1044)
Moderate	<0.5µg/mL	03	03	0.6268		
	>0.5µg/mL	05	00			
Mild	<0.5µg/mL	21	02	0.326	0.568	2.100
	>0.5µg/mL	14	02			(0.1224 to 20.36)

Table 16. Association of ferritin levels with CXCR3 (rs779120264) gene polymorphism and COVID-19 cases

Severity of COVID	Serum Ferritin	AA	AC+CC	Chi Square value	P VALUE	OR (95% CI)
Severe	<464ng/mL	13	01	0.4384	0.5079	0.3939
	>464ng/mL	33	01			(0.02022 to 8.021)
Moderate	<464ng/mL	05	01	0.900	0.3428	0
	>464ng/mL	03	02			(0 to 11.25)
Mild	<464ng/mL	26	01	1.415	0.234	4.375
	>464ng/mL	12	03			(0.2451 to 42.24)

Table 17. Association of neutrophil count with CXCR3 (rs779120264) gene polymorphism and COVID-19 cases

Severity of COVID	Neutrophils	AA	AC+CC	Chi Square value	P VALUE	OR (95% CI)
Severe	<80%	08	00	0.4174	0.5182	0
	>80%	38	02			(0 to 0.00899)
Moderate	<80%	04	1	0.2444	0.6210	2.000
	>80%	04	02			(0.1640 to 35.37)
Mild	<80%	16	03	3.748	0.0529	0
	>80%	22	00			(0 to 0.9417)

results were in accordance with a meta-analysis study by Huang et al.[22] One hypothesis states that the virus instantly targets and kills, cells that express the SARS-CoV-2 receptor ACE2. Another theory is that in COVID-19, high levels of pro-inflammatory cytokines such as tumor necrosis factor (TNF) and IL-6 encourage lymphocyte apoptosis.[23] Our study showed no significant

Table 18. Association of SGOT levels with CXCR3 (rs779120264) gene polymorphism and COVID-19 cases

Severity of COVID	SGOT	AA	AC+CC	Chi Square value	P VALUE	OR (95% CI)
Severe	<59	23	02	1.920	0.1659	0
	>59	23	00			(0 to 2.322)
Moderate	<59	05	01	0.7486	0.3869	3.333
	>59	03	02			(0.2586 to 56.53)
Mild	<59	29	02	0.1404	0.7079	1.611
	>59	09	01			(0.1009 to 14.93)

Table 19. Association of ESR levels with CXCR3 (rs779120264) gene polymorphism and COVID-19 cases

Severity of COVID	ESR	AA	AC+CC	Chi Square value	P VALUE	OR (95% CI)
Severe	<20	05	00	0.2427	0.6223	0
	>20	41	02			(0 to 0.05072)
Moderate	<20	00	00	P value could not be calculated as		
	>20	08	03	sample size was 0		
Mild	<20	16	01	0.08815	0.7665	1.455
	>20	22	02			(0.1572 to 22.25)

Table 20. Association of RBS levels with CXCR3 (rs779120264) gene polymorphism and COVID-19 cases

Severity of COVID	RBS	AA	AC+CC	Chi Square value	P VALUE	OR (95% CI)
Severe	<140 mg/dL	16	00	1.043	0.3070	0
	>140 mg/dL	30	02			(0 to 0.2310)
Moderate	<140 mg/dL	01	02	3.227	0.0724	0.07143
	>140 mg/dL	07	01			(0.004481 to 1.409)
Mild	<140 mg/dL	24	01	0.7691	0.3805	0.2917
	>140 mg/dL	14	02			(0.01486 to 6.282)

Table 21. Association of Hb% levels with CXCR3 (rs779120264) gene polymorphism and COVID-19

Severity of COVID	Hb%	AA	AC+CC	Chi Square value	P VALUE	OR (95% CI)
Severe	<15	41	02	0.2427	0.6223	0
	>15	05	00			(0 to 19.72)
Moderate	<15	07	03	0.4123	0.5207	0
	>15	01	00			(0 to 24.00)
Mild	<15	24	02	0.01475	0.9033	0.8571
	>15	14	01			(0.05557 to 7.915)

association of lymphocyte level with disease severity. Increased neutrophil percentage was observed in severe COVID cases which was in accordance to many studies. In response to infection neutrophils release neutrophil extracellular trap, cytokines and produce reactive oxygen species that help combust infection. This explains the increase of neutrophils in COVID-19.[24]

A meta-analysis study comprising of nine research papers involving 1779 COVID-19 patients showed thrombocytopenia in COVID-19 infection thus indicating the advancing illness while hospitalized.[25] In our study, 24% and 21.5% decrease in platelet count was observed in severe COVID cases compared to mild and moderate cases.

Role of acute phase reactants with severity of COVID-19 infections

Zhou et al stated that increased D-dimer is a separate risk factor for disease progression and is markedly elevated in instances of severe pneumonia. Our study supported this observation as elevated d D-dimer levels, more than the normal range were observed in severe COVID cases (1.305 microgram/mL) with a highly increased significant association of D dimer in these patients. Increased D-dimer indicates an imbalance between pro-inflammatory and anti-inflammatory cytokines and a surge of cytokines that results in severe damage to endothelial cells that can cause hypercoagulation, disseminated intravascular coagulation, and acute respiratory distress in COVID-19.[26]

Cytokine storm, numerous inflammatory triggers, cellular damage, metabolic acidosis and associated release of ROS in COVID-19 illness release ferritin from macrophages in lung parenchyma. In acute as well as chronic inflammatory reactions, the acute phase reactant serum ferritin is released.[27] Hence serum ferritin levels was increased significantly in severe COVID cases in our study and an association between increased serum ferritin level and severe COVID-19 disease. Serum ferritin was thus found to be an independent predictor of severe SARS-CoV-2 disease.[28-30] Our study has also reported that patients with severe COVID-19 showed notable elevations and significant association with ESR.

Similarly, ESR was significantly elevated in serious COVID-19 patients in ICU with oxygen support as compared to other COVID cases. ESR which is also a measure of inflammation is not specific and can increase in various other pathological conditions.[31] Perhaps making ferritin and D dimer the trademark for disease severity in COVID-19 cases.

CONCLUSION

Our study demonstrated significant associations between elevated serum D-dimer, ferritin, RBS, neutrophil count, ESR, and hemoglobin levels in severe COVID-19 cases compared to mild cases. Lymphocyte and platelet count also varied significantly with disease severity, suggesting they may be more reliable predictors in distinguishing between moderate and severe COVID-19 cases. No significant associations were observed between gene polymorphisms and markers of disease severity, except for the wild-type CXCL10 genotype, which was linked to increased ferritin levels. This suggests that mutations in CXCL10 may provide protective effects compared to the wild-type variants.

Although CXCR3 gene polymorphisms were prevalent in a significant number of patients, no significant associations were observed between CXCR3 polymorphisms and biochemical or hematological markers. These findings suggest that while CXCL10 polymorphisms may influence the severity of COVID-19, CXCR3 polymorphisms may not have a direct impact on clinical outcomes. Further research is required to understand the role of these genetic variations in the progression and severity of COVID-19.

CONFLICT OF INTEREST
The authors declare that there is no conflict of interest.

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

FUNDING

DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

This study was approved by the Central Ethics Committee, Nitte (Deemed to be University) with protocol number INST.EC/EC/110/2021-22.

INFORMED CONSENT

Written informed consent was obtained from the participants before enrolling in the study.

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