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Original Article

Comparison of Interferon-Inducible Patient-10 and Chemokine Ligand-2 Expression in COVID-19 Patients

Nayab Khalid, Mehwish Qamar, Sadia Syed*, Ibrahim Liaqat, Muhammad Irfan ul Akbar Yousufzai, Summyah Niazi*****

Department of Physiology, Islam Medical and Dental College, Sialkot, Pakistan, *Department of Forensic Medicine & Toxicology, Shifa College of Medicine, Islamabad, Pakistan, **Department of Physiology, Sahara Medical College, Narowal Pakistan, ***Department of Physiology, Quetta Institute of Medical Sciences, Quetta Pakistan

ABSTRACT

Objective: To compare interferon-inducible patient-10 and chemokine lisgand-2 expression in COVID-19 Patients.
Study Design: Cross-sectional study.
Place and Duration of Study: University of Lahore Teaching Hospital, Lahore Pakistan, from Dec 2020 to Mar 2021.
Methodology: We assessed the level of cytokines in forty-five COVID-19 patients and compared it with the 45 healthy individuals. The pro-inflammatory cytokines IP-10 and CCL-2 levels were evaluated in patients and healthy people via ELISA kits.
Results: The study included ninety (n=90) patients. Our data suggested that the serum levels of IP-10 and CCL-2 were increased (8.5±1.5 ng/ml and 11.89±4.55 pg/ml) significantly in patients with COVID-19 as compared to healthy Controls (4±0.5ng/ml and 6.15±1.91 pg/ml). A significant increase was recorded in INR, PT (sec) and APTT (sec) levels in COVID-19 patients. Whereas the platelets count (109/ml) was decreased significantly in COVID-19 patients (220±42) as compared to healthy individuals (419±69). Significant elevation of D-Dimer (ng/ml) was recorded in patients with COVID-19.
Conclusion: Our data suggested a significantly increased level of IP-10 and CCL-2 in COVID-19-associated patients, which may be of essential diagnostic and prognostic significance for these biomarkers.

Keyword: COVID-19, ELISA test, Inflammatory cytokines.

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INTRODUCTION

Coronavirus belongs to the alpha and beta groups of Coronavirus (MERS CO-V and SARS CO-V).[1] At the initial phase of SARS-CoV and MERS CoV disease, the dendritic cells, macrophages, and respiratory epithelial cells, the release of IFN-1 and IFN-α/β, primarily trailed by cumulative stages of pro-inflammatory cytokines as the disease develops.[2,3] The inflammatory infiltration of lung tissue by monocytes and neutrophils is due to the rapid surge in pro-inflammatory cytokines, which are the pillars to mediate lung injury.[4] In addition, the raised levels of pro-inflammatory cytokines excite T-cell apoptosis and interrupt viral clearance.

Current revisions have revealed the COVID-19 disease brutality, which triggers a "cytokine storm syndrome." It is a hyperinflammatory syndrome categorized by fulminant and lethal hyper-cytokinemia.[5,6] In these "cytokine storm syndromes," there are increased interleukins, including IL-2, IL-7, granulocyte-colony stimulating factor, interferon-γ inducible protein-10, monocyte chemoattractant protein 1, macrophage inflammatory protein 1-α, and tumour necrosis factor-α are present. This indicates the severity of infection and expansion in this lethal COVID-19 disease.[7] The reduction of antiviral defences in COVID-19 could relate important points to the innate immune response and the manufacture of inflammatory cytokines.[8] An up-regulation of the cytokine concentration in cytokine syndromes such as IL-1ß, IP-10, IFN-γ, and MCP-1 central to the initiation of Th1 cell response, which future worsens the deadly cytokine storm that happened in SARS-CoV and MERS-CoV infection. Besides the immunological responses, recent studies have compared cruelly ill and non-severely ill patients.[9] There may be meaningfully increased levels of IL-2, IL-7, IL-17, IL-10, MCP- 1, MIP-1A, and TNF-α, indicative of speckled cytokine profile in between the two groups and also the participation of cytokine storm in disease harshness as well as disease evolution.[10]

Recent studies on coronavirus-infected patients' clinical and laboratory features have been published, especially in the Chinese population. However, the

Correspondence: Dr Sadia Syed, Department of Forensic Medicine & Toxicology, Shifa College of Medicine, Islamabad, Pakistan
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clinical trials of that lethal disease still need to be conducted in different populations across the border. The current study aimed to assess interferon-inducible protein-10 and chemokine ligand-2 expression levels in COVID-19 patients.

METHODOLOGY

The cross-sectional study was conducted at the University of Lahore Teaching Hospital, Lahore Pakistan, from December 2020 to March 2021 after approval from the Internal Review Board and Ethical Committee.

Inclusion Criteria: Patients of either gender, aged above 18 years, who were PCR positive for COVID-19 infection, admitted in isolation rooms or wards of the hospital with maintaining oxygen saturation on room air requirement less than 5 litres of non-invasive oxygen, were included.

Exclusion Criteria: Patients suffering from other lung diseases, patients who were admitted to isolation wards on suspicion and having PCR negative for COVID-19 infection, were excluded.

The study included a total of ninety (n=90) patients. They were further divided into two groups. Group-A included forty-five healthy individuals as a Control-Group, and Group-B included diagnosed cases of COVID-19 infection by polymerase chain reaction (PCR). To collect blood samples, venipuncture was performed, and 5 ml of blood was drawn from each participant and transferred to the test tube. ELISA test was performed. Serum was obtained by centrifugation of 5 mL whole blood sample and stored at −80°C until further use. The sample collection was performed when the patient's condition became severe and entered the ICU. At this time, experimental testing and clinical laboratory data were collected. The amount of three inflammatory cytokines, such as IP-10 and CCL - 2 (all from Abcam Ltd., Cambridge, UK), was measured in the serum using the human enzyme-linked immunosorbent assay (ELISA) kit (Abcam). The assay was performed according to the manufacturer's instructions.

Statistical Package for Social Sciences (SPSS) version 25.0 was used for the data analysis. Quantitative variables were expressed as Mean±SD and qualitative variables were expressed as frequency and percentages. Independent sample t-test and the Chi-square test were applied to explore the inferential statistics. The *p*-value of 0.05 or less was taken as significant.

RESULTS

The study included ninety (n=90) patients. Females suffering from COVID-19 and admitted to the hospital were found to be significantly older (*p*-value=0.03), with a mean age of 52.53±10.61 years. Fifteen per cent of the males were smokers (Table-I).

Table-I: Demographic Distribution in COVID-19 Patients (n=45)

Variables	Male Patients (n=27)	Female Patients (n=18)	*p*-value
Age*–years (Mean±SD)	42.35±9.67	52.53±10.61	0.03***
BMI*–kg/m2	26.47±7.52	24.91±4.30	0.42
Smokers History**n(%)	5(19)	1(11)	0.04***
Diabetes** no(%)	4(14.8)	3(16.7)	0.67
Hypertension**–no(%)	12(44.4)	9(50)	0.31

Comparison was done by using the independent sample T-test Comparison was done by using the chi-square test***The p-value was statistically significant*

We evaluated the level of cytokines in two different groups. One group included the COVID patients that PCR confirmed, while the second group had healthy individuals. Our data suggested that the level of cytokines, IL-1, IL-6, and IL-8 significantly differs in the disease group compared to the healthy individuals. Highly increased levels of these cytokines were observed in COVID patients relative to healthy individuals. In addition, we assessed the serum levels of different interferons, including IP-10 and CCL-2, that were significantly increased in COVID-associated patients relative to the healthy group. The level of IP-10 was 4±0.5ng/ml in patients, while the level of IP-10 was 8.5±0.05 ng/ml in the Control Group group. On the other hand, the concentration of CCL-2 was 11.89±4.551 ng/ml in patients compared to 6.15±1.89 ng/ml in the Control Group (Table-II).

Table-II: Comparison of Different Prophetic Variables in COVID-19 Patients

Variables	Control Group (n=45)	COVID-19 Patients (n=45)	*p*-value
Interleukine-1 (IL-1)– pg/ml	10.34±1.5	15.23±0.8	0.07
Interleukine-6 (IL-6)–pg/ml	5±0.9	8.5±0.05	0.05*
Tumor necrotic factor-α (TNF- α)–pg/ml	230±0.8	350±0.8	0.02*
Interferon-inducible patient-10 (IP-10)–ng/ml	4±0.5	8.5±1.5	<0.001*
Chemokine ligand-2(CCL-2)–pg/ml	6.15±1.91	11.89±4.55	<0.001*
International normalized ratio (INR)	1.1±0.1	3.5±1	<0.001*
Prothrombin time (PT)– sec	10±0.02	16.2±2.93	0.03*
Activated partial thromboplastin (PTT) – sec	28±0.12	48±1.0	0.01*
Platelets–109/ml	419±69	220±42	<0.001*
D-Dimer–ng/ml	125±0.7	887±22	<0.001*

**The p-value was statistically significant*

DISCUSSION

This study aims to understand human coronavirus infection with lethal respiratory syndrome pathology and the key role of innate and adaptive immunity in COVID-19-infected patients. Identifying the deviating response and mechanism is urgently demanded in developing immune-modulating therapies.

We reported that the number of female subjects was lower than males in the present study for the demographic distributions. The females admitted to the hospital in our study were significantly older than the males. Similar findings were reported in review articles by Kopel Jonathan *et al*. and Beltrame *et al*. where they concluded that higher estrogen levels in females lead to increased innate and humoral immunity compared to males.[11,12]

In our study, we found a significant difference in the smoking status between male and female patients. This difference might be due to the social norms prevailing in our society. Similarly, a Turkish study also reported an association between smoking and the severity of the disease in the male gender. Gene expression and subsequent receptor levels are elevated in the airway and oral epithelium of current smokers, consequently putting smokers at higher risk.[13]

When the COVID-19 patients were compared among the healthy controls in the study under discussion, we found significant differences between all the cytokines except IL-1. Evidence reports that chemokine selectively recruits neutrophils, monocytes, and lymphocytes in response to any infection by inducing chemotaxis through the activation of G protein-coupled receptor, as in the case of coronavirus infection. Data from different retrospective studies have shown various chemokines, including plasma concentration of IL2, IL6, IL8, IL10, IL17, IP10, CCL2, Interferon-gamma, monocyte chemoattractant peptide, and macrophage inflammatory protein 1, are up-regulated in COVID 19 patients. Further analysis demonstrates that serum levels of IL-2, IL-7, IL-17, 1L-10, MCP-1, MIP-1A, and TNF-alpha are raised in ICU patients more than in non-ICU symptomatic patients, causing lymphopenia.[14,15] Moreover, three chemokines, including IP-10, IL-10, and IL-7, are raised in asymptomatic patients. In response to the inflammatory cascade, cells from the air spaces secrete these inflammatory mediators (pro-inflammatory cytokines and chemokines). Additionally, IP10 shows correlation and impacts other cytokines IL-6, Interferon-gamma, and intercellular adhesion molecule-1.[16]

CCL2 is a potent chemokine that participates in the immune response during inflammation, such as macrophage recruitment and polarization. It is secreted by smooth muscle cells, epithelial cells, astrocytes, meningeal cells, and multiple pulmonary cells. CCL2, by regulating the recruitment of immune cells, especially macrophages, and during inflammation, causes polarization and affects various immune cells. CCL2 causes chemotaxis of monocytes, myeloid and lymphoid cells. CCL2 mainly affects monocytes because its receptor, CCR2, is expressed in higher monocyte concentrations.[17] Like other chemokines, activation of its receptor elicits several downstream cascades, like JAK2/STAT3 signalling, PI3K signalling, and MAP kinase signalling. These signalling cascades are involved in the migration process of immune cells and phospholipase C-mediated calcium release.[18] Evidence has cleared that the inflammation is caused in response to migration and infiltration of monocytes from the bloodstream across the vascular endothelium, influencing cellular adhesion, polarization, cellular survival, and autophagy aid in the disease progression. CCL2 expression is markedly increased in response to inflammatory stimuli. In the case of proteoglycans, CCL2 also interacts with glycosaminoglycan. Hence, CCL2 exhibits a multitude of effects. Moreover, knockout of CCL2 has shown decreased lipid body formation. Surprisingly, CCL2 hosts chemotaxis by targeting the immune cells and shows the effector functions from cellular cleanups to its metabolism and allergic responses.[19] These responses in infectious diseases like Coronavirus have given CCL2, especially protocol for the urgent need for new treatment drugs.

RECOMMENDATIONS

Detailed histological and autopsy findings in the dead subjects and their correlation with IP-10 and CCL-2 should be established.

CONCLUSION

Our data suggested a significantly increased level of IP-10 and CCL-2 in COVID-19-associated patients, which may be of essential diagnostic and prognostic significance for these biomarkers.

Conflict of Interest: None.

Authors' Contributions

The following authors have made substantial contributions to the manuscript as under:

NK &MQ: Concept, statistical analysis, data interpretation, critical review, approval of the final version of the manuscript

SS & IL: Data acquisition, data analysis, critical review, approval of the final version to be published and correspondence

MIUAY & SN: Study design, data interpretation, drafting the manuscript, critical review

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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