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GENE EXPRESSION OF IL-10 IN COVID-19 PATIENTS

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Abstract

Background: *In December 2019, an epidemic viral disease spread which later converted into a pandemic viral disease, this viral infection is known as COVID-19. Severe Acute Respiratory Syndrome Coronavirus 2 is the pathogenic and highly transmissible virus that causes COVID-19 disease. Pakistan is one of the major countries affected by SARS-CoV-2 with fatal consequences.*

Objective: *The purpose of this investigation was to investigate the expression of interleukin-10 in healthy, severe to critical, and COVID-19 mild to moderate sufferers by ELISA and RT-PCR.*

Methods: *The study was conducted among 150 adult patients with COVID-19 (Classification as mild/moderate and severe patients. Serum samples were tested for IL-10 levels and expression by ELISA and Real-time PCR. The statistical analysis was performed using Graph Pad prism software and the Kruskil Walis test was applied.*

Results: *The median levels of IL-10 in healthy, mild/moderate, and severe patients are 6.45, 6.78, and 7.44. While the p-value is insignificant (p 0.269). The expression of IL-10 was studied in the peripheral blood of patients with severe COVID-19 compared to mild/moderate symptomatic COVID-19 and healthy individuals. In the peripheral blood of COVID-19 patients, the level of IL-10 mRNA was 6.024 in severe cases and 4.58 in mild to moderate subjects (1). Notable differences were noted in the expression of interleukin-10 in the circulating cells of sever COVID-19 patients compared to those with mild cases and the control group.*

Conclusion: *In conclusion, IL-10 levels and expression are higher in severe and mild/moderate patients as compared to healthy individuals which may contribute to the pathogenicity of COVID-19 patients.*

INTRODUCTION

SARS-CoV-2 is a coronavirus that causes an extremely transmittable disease called COVID-19 (1). Mild to moderate symptoms are shown by a large number of patients, severe pneumonia occurs roughly in 15% of the victims of COVID infection, and acute respiratory distress syndrome (ARDS), septic shock and multiple organ failure develop in 5% of these victims (2). The signs & symptoms of COVID-19 infection occur after the incubation period (5-7 days). The symptoms like flu, runny nose, cough, fever, headache, shortness of breath, diarrhoea, shortness of breath, and lymph node shrinkage are the consequences of COVID-19 on human physiology. The CT test of the chest indicated pneumonia as a distinctive feature of COVID-19 infections, acute respiratory distress syndrome and acute myocardial infarction presence which caused demise (5). Coronaviruses own the leading genomes among recognized RNA viruses. Coronaviruses are single-stranded RNA (ssRNA) within the range of 26 to 32 kb in length non-segmented and enveloped viruses (6).

COVID-19 pathogenesis starts with viral inclusion through its target host cell receptors. According to numerous researchers SARS-CoV-2 utilized receptor angiotensin converting enzyme 2 (ACE2) in humans. Large respiratory droplets, are cause of transmission of SARS-CoV-2, which infected the upper and lower breathing tract cells. Against SARS-CoV-2 contamination the first physiological defence is Immune response (7)

In severe COVID-19 diseased patients cytokine storm is triggered in which immune cells are highly activated and inflammatory cytokines and chemical mediators are producing in excessive amount which cause severity of disease, high levels cytokines, lymphopenia and death in COVID patients. Level of Interleukin 1β, 10, 6, 8, 12, 7, IP-10, MCP-1, TNF-α, macrophage inflammatory protein 1 alpha increase with severity of disease (8). The continuous interleukin 10 (IL-10) activity in coronavirus ailment 2019 (COVID-19) is a particular characteristic of the cytokine storm. To suppress inflammation this notion became a negative feedback mechanism. However, numerous traces of medical proof recommend that in pathological aspect of COVID-19 severity proinflammatory, IL-10 elevation can also play a role (9). The immune regulatory cytokine interleukin-10 (IL-10) play an essential action to suppress and terminating inflammatory immune responses, in large part via the inhibition of monocyte and macrophage activation (10)

IL-10 is a significant anti-inflammatory immune-modulating agent. The negative feedback loop of proinflammatory cytokines embark the raised release of IL-10 in COVID-19 patients (10), based on its immunoregulatory and anti-inflammatory response, the recombinant IL-10 can be used as a treatment modality. Now, research studies showed the raised levels of IL-10 in SARS-CoV-2 infection can have detrimental effects on the pathophysiology of COVID-19 severity.

The purpose of the study was to look at the expression and amount of interleukin -10 in individuals with COVID-19 who fell into two categories: mild/moderate and severe. Reports indicate that individuals with severe COVID-19 display higher levels of the cytokine IL-10 compared to those with mild or moderate infections highlighting that IL-10 may be involved in pathophysiology of disease.

MATERIALS AND METHODS

In this comparative study, healthy individuals and confirmed COVID-19 patients were included with their written consent forms. Samples were taken from Mayo Hospital, the UHS COVID lab, University of Veterinary & Animal Sciences (UVAS). The duration of the study was 06-months.

In this study we compare the gene expression of interleukin 10 of healthy individuals with COVID-19 victims. The sampling technique was a non-probability purposive sampling technique. The study group consisted of 150 subjects (Both Gender). A total of 150 COVID-19 patients' age and gender-matched healthy individuals from the same geographical and ethnic origin participated in this study. COVID-19 patients in this study were by RT-PCR. These study subjects were categorized into three groups based on WHO clinical classification of COVID-19 patients. One hundred and fifty patients were divided into 3 groups. Healthy individuals were included in group 1 and subjects with mild to moderate COVID-19 were inducted in group 2.

Patients who exhibited symptoms of respiratory frequency greater and equal to 30/min, dyspnea, pneumonia, blood oxygen saturation (SPO2) ≤ 90%, PaO2/FiO2 ratio (the ratio of blood pressure of oxygen and the percentage of oxygen supplied PaO2) < 300 and if lung infiltrates > 50% within 24 to 48 hours were classified as severe COVID-19.

Blood was divided in two tubes EDTA and gel tube (Citrate or sodium heparin). Through an ice box, samples were transported to the Department of Immunology. The samples were centrifuged at 800g, 4 C for 10 min and the supernatant was stored at -80 C. While for PCR blood vials with EDTA were subjected to RNA isolation.

ELISA (enzyme-linked immune sorbent assay) was performed for the detection of IL-10 levels. For this procedure, a fine test kit was used. For real-time PCR first trizol method was used to extract the RNA in this method after trizol addition we use following chemical in respective order chloroform, isopropyl for extracting and for washing ethanol was used. RNA was used for synthesis of cDNA, Thermo Scientific Revert Aid First cDNA Synthesis Kit was used for this process. Cellular gene expression investigation was carried out by introducing certain cellular gene primers to real time PCR (RT-PCR) using RT-Qpcr master mix in accordance to protocol's instruction of thermo scientific kit.

Graph pad Prism software was used for the analysis of the data. Results were analysed by SPSS Software, mean and standard deviation along with t-test were implemented. Kruskal-wallis test was applied for group comparison.

RESULTS

In this comparative observation study 150 participants were recruited, who were assigned to three different groups. In group 1, healthy individuals were assigned. Group 2 and group 3 consisted of mild to moderate and severe to critical COVID-19 patients respectively. In group 1, 50% (n=25) subjects were male and 50% (n=25) were female. In group 2 the ration of male and female was 70:30% and in group 3 it was 60-40%. (Table: 1.1). Male are more prone to the COVID-19 disease.

After serum separation quantitative detection of IL-10 was done by using Fine Test kit. Then it is place in microplate reader and reading was taken at UV 450 nm IL-10 was determined by mean value and for analysis of data Graph Pad Prism software was used. Markedly increase expression was observed in mild symptoms COVID-19 patients and severe COVID-19 patients. The median level of Interleukin -10 in serum of control (group-1) was 6.45, in mild to moderate symptomatic patients it was were 6.78 and in severe to critical symptomatic patients was 7.44. The highly significant differences were observed in quantity of interleukin-10 between these groups. As the graph pad analysis is followed by Kruskal-Wallis test it was observed to be insignificant (p=0.26). (Table 1.2) (Figure 1.1). While the median level of IL-10 was higher in severe and moderate patients as compared to the healthy individuals.

Table 1.1: Epidemiological data of COVID-19 Patients ELISA Assessment

Variables	Total n=50	Mild/Moderate n=50	Severe n=50	*p* value
Gender				
Male	30	35	30	N/A
Female	20	15	20	

Table 1.2: Interpretation of IL-10

	Median	Interquartile range	Independent-samples Kruskal-Walli's test
Healthy individuals	6.45100	4.85825- 8.86550	0.269
Mild symptoms with COVID-19 disease	6.78600	5.72200- 13.96825	
Severe symptoms		6.20175- 14.33575	

with COVID-19 disease	7.44350		

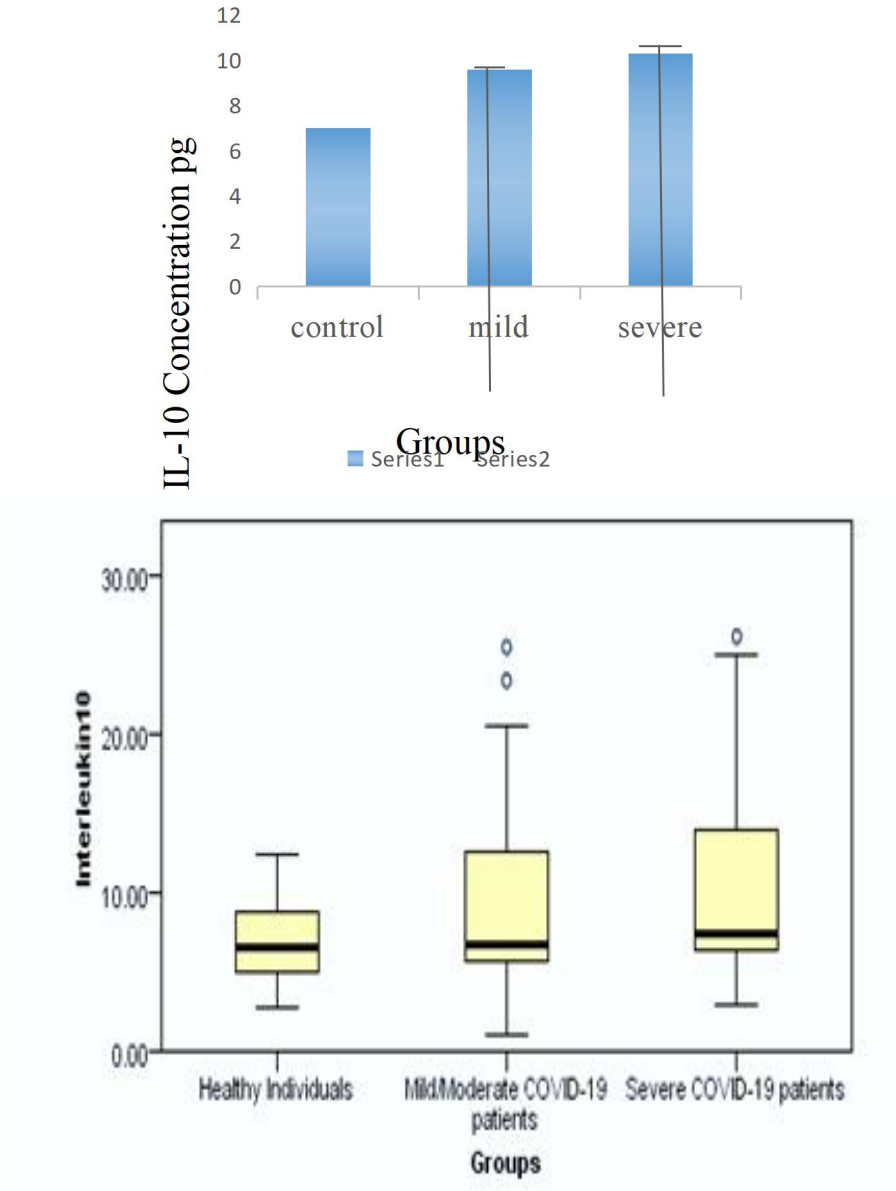


Figure 1.1: Column graph representation of IL-10 gene expression average value in healthy COVID-19 mild and severe symptomatic groups

Expression of Interleukin-10 in COVID-19 patients by RT-PCR

RNA was isolated from blood by Trizol method. CDNA was made by using a thermo kit. IL-10 forward and reverse primers were used to amplify the product using real-time PCR. Gene expression was determined, and normalized to constitutively active gene Interleukin-10. The IL-20 values were expressed as fold change to compare with a mean value of control. Patients with severe COVID-1 symptoms had circulatory peripheral blood interleukin-10mRNA levels of 6.024 and patient with mild to moderate COVID-19 symptoms had a value of 4.58 (1) (Table: 1.3 and 1.4). Highly significant differences were observed in the expression of interleukin-10 in circulatory cells of severe COVID-19 as compared to the mild and control group (Fig: 1.2 and 1.3).

Table 1.3: Gene expression value of control, mild COVID-19 and Severe COVID-19 Patients by Real Time PCR

Sample no.	Control	Mild	Severe
1	1	3.5	4.5
2	1	4.5	5.5
3	1	3.3	5.2
4	1	3.8	4.3
5	1	4.5	5.6
6	1	5.5	6.4
7	1	5.3	6.8
8	1	5.5	7.2
9	1	5.6	6.8
10	1	4.3	6.9
11	1	3.6	4.7
12	1	4.3	5.3
13	1	3.4	5.4
14	1	3.7	4.6
15	1	4.4	5.8
16	1	5.2	6.2
17	1	5.4	6.9
18	1	5.6	7.0
19	1	5.3	6.4
20	1	4.4	7.1
21	1	3.9	5.7
22	1	3.5	5.1
23	1	4.9	6.05
24	1	5.01	6.8
25	1	4.8	6.7
26	1	3.8	4.8

Table 1.4: Gene expression value of control, mild COVID-19 and severe COVID-19 Patients by Real Time PCR

Sample no.	Control	Mild	Severe
27	1	5.56	5.3
28	1	4.6	6.6
29	1	5.6	6.03
30	1	4.7	6.7
31	1	5.5	5.1
32	1	4.8	4.8
33	1	3.8	5.4
34	1	5.31	7
35	1	4.3	5.9
36	1	3.9	5.4
37	1	5.41	6.5
38	1	3.3	6.3
39	1	4.9	6.4

40	1	5.6	5.7
41	1	3.4	6.9
42	1	5.4	5.8
43	1	4.4	7.1
44	1	4.7	6.6
45	1	3.8	6.8
46	1	4.2	7.02
47	1	5.2	6.2
48	1	4.6	6.4
49	1	4.4	6.0
50	1	4.9	5.5
Mean value	1	4.5858	6.024

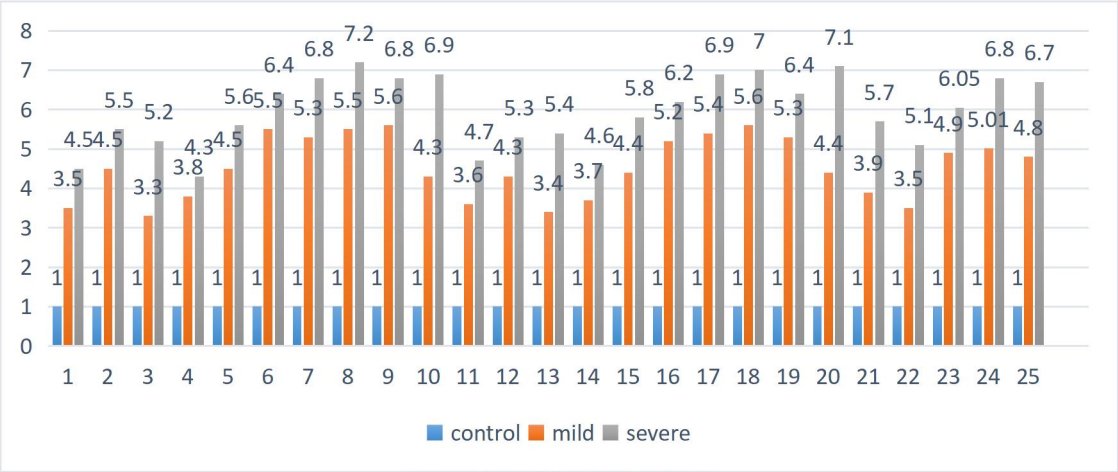


Figure: 1.2: Expression of IL-10 in control, mild cases and severe case of COVID-19

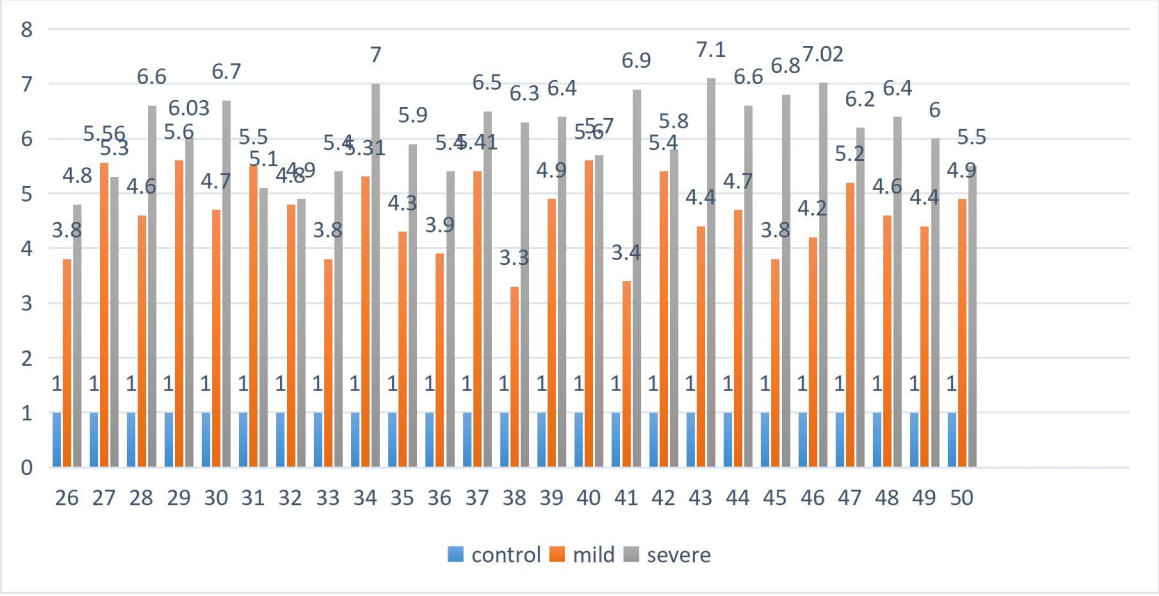
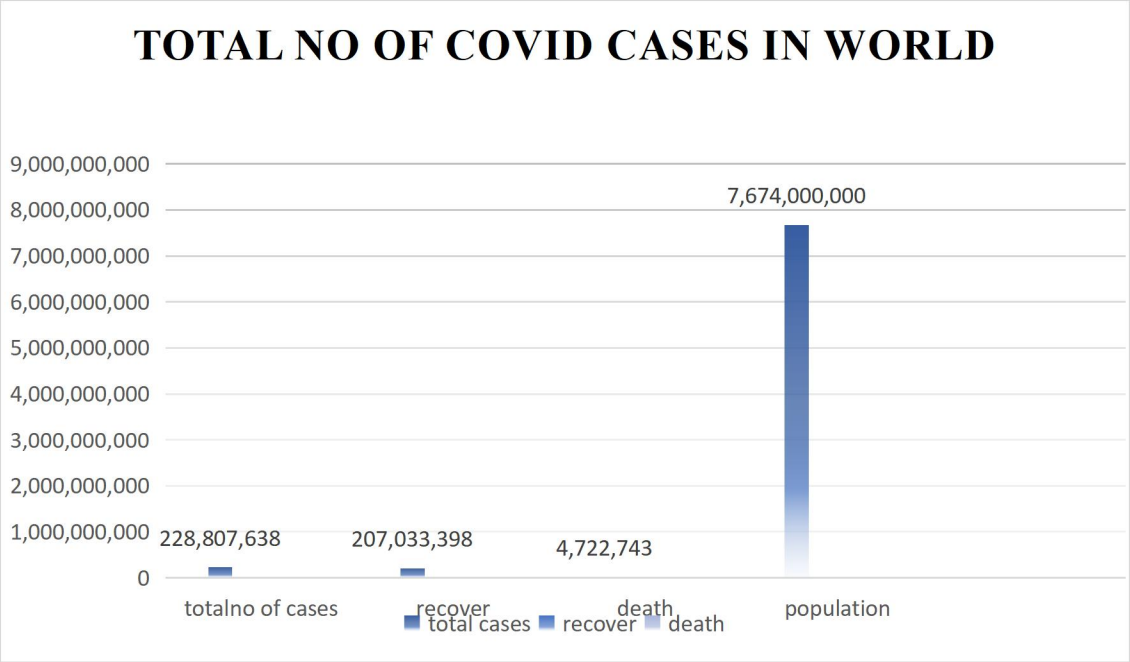


Figure: 1.3 Expression of IL-10 in control, mild cases and severe case of COVID-19



Discussion

The SARS-Cov-2 emerge in December 2019. In this twenty first century third major coronavirus. March 11, 2020, a dreadful day when coronavirus has transmit promptly internationally result in COVID-19 ailment caused by coronavirus to become pandemic (11). In Pakistan first corona victim has appeared in Karachi on February 26, 2020. Currently, infectious individuals attain a range are almost 1.22 million, in addition to this, the demise value is 27 thousand with death ratio last week almost reaching 10% (12). Pakistan has higher ratio of men contamination almost 6.7% and a lower ratio of women contamination almost 3.6% as reported by the National health services Ministry (13).

Interleukin-10 is a well-known anti-inflammatory cytokine, and it has numerous crucial functions. Suppression of dendritic cell and macrophage, stimulation and restrictions of Th1 and Th2 effector reactions, during contamination. Ensued defence of the host through restraining destruction of tissue instigated by current inflammation on the other hand stimulates contamination perseverance. The power of foregoing inflammation reflected by strength of IL-10 reaction. IL-10 is possibly up regulated in COVID-19 during overpowering inflammation, similarly, subsidize in progress of lung fibrosis and inflammatory cells intrusion in lungs (14). Decreasing or straight ending inflammatory reactions in cytokines of proinflammation was influence of IL-10 (15).

IL-10 has both proinflammatory and anti-inflammatory effects. Nevertheless, IL-10 can have immunostimulatory effects, such as promoting CD8+ T cells' production of IFNγ. It also plays a significant role in the development and differentiation of mast cells, thymocytes, and B cells. Similar to other cytokines, this interleukin has been found by several authors in COVID-19 patients and has been linked to the severity and course of the illness. It may also have predictive value. In fact, studies suggested that IL-10 expression is increased in SARS-CoV-2 infection, and it is higher in patients with a hyperinflammatory response. In conclusion, patients with COVID-19 had higher levels of IL-10 than did those with SARS-CoV or MERS. (16)

COVID-19 severity and unfortunate results are considerably related to IL10 and IL1Ra. Therefore, in severely sick sufferers compromised Type I IFN making occurs which do not hinder virus-prompted IL-10 manufacturing that additionally weakens antiviral reactions. IL-10 and similarly IL-1Ra were expressed greatly in infested victims of SARS-CoV in comparison with control, predominantly deceased severely sick sufferers, establish with an earlier observation. Therefore, consequential compromised type I IFN reactions was due to up regulation of IL-1Ra IL-10 as a result of negative response of anti-

inflammatory cytokines (9). Elevation of IL-10 occur in critical cases; thus, IL-10 inhibitory attribute probably promotes immune system repression, ailment severity, and viral control (17)

Moreover, throughout hospitalization of victim's blood levels of IL-10, sTNFRSF1A, IL-15, and sST2, make difference between fighters from deceased, they appeared as 3 different biomarkers. NK cell development and function, also in inflammatory reactions IL-15 was credited as main character. Probably feasible tactic for COVID-19 is IL-15 immunotherapy. Correlation of elevated IL-10 blood intensities and illness harshness and evolution, in COVID-19 victims, have been discuss in numerous observations. Occurrence which have been witnessed in myeloid cells of severe COVID-19 sufferers is IL-10 suppress HLA class II molecules expression through antigen-presenting cells. Additionally, a substantial increase in regulatory T cells which produce IL-10 were perceived in blood of severe COVID-19 sufferers, in comparison with mild and moderate illness. Lung inflammation is control by IL-10–manufacturing regulatory T cells which show perilous function through confining progress of Th17 cells that cause tissue-destruction and constraining reactions of innate inflammation. For neutralize severe inflammation of lung elevated IL-10 quantity in victims of COVID-19 who has expired, perceived in this observation. Possible in COVID-19 sufferers elevated IL-10 might subdue antiviral adaptive immune reactions and decline fight with bacterial super contaminations in COVID-19 victims. The sTNFRSF1A is a biomarker which was related to fatality of victims (9).

Tocilizumab, attach with IL-6 receptor with extraordinary attraction, it work like reunification sophisticate receptor of anti-human IL-6 monoclonal antibody, consequently precluding IL-6 from attaching with its own receptor, delineate IL-6 for immune destruction of target cells, and lessening inflammatory reactions (17)

In this study, there was highly significant difference in case of interleukin-10 expression in circulating blood of severe COVID-19 patients as compared to mild and control groups.

The significance of gene expression difference between groups was evaluated by Kruskal-Wallis test. To ascertain whether there are statistically significant differences between two or more groups of an independent variable on a continuous or ordinal dependent variable, a nonparametric test based on rank might be employed. It is considered the nonparametric alternative to the one-way ANOVA.

The analysis of IL-10 gene expression was done by serum in ELISA and by RT-PCR. The statistical analysis was done by Graph pad Prism which shows highly significant relationship between interleukin-10 and COVID-19 disease. The statistical analysis between interleukin-10 and COVID-19 disease shows highly significant association between groups that confirm the effect of interleukin in COVID-19 disease in other studies. The amplification products were quantified in absolute terms using the standard curve method, and the specificity was confirmed through the examination of a melting curve. By serially diluting known concentrations of the corresponding cDNA gene template, standard curves for the expression of each gene were produced.

Our study suggests that IL-10 influences the severity of COVID-19 disease (10). All in all, these results suggest that interleukin-10 cooperatively plays role in the severity of COVID-19 disease regardless of age. To our knowledge there are research on interleukin -10 cytokines in relation to the severity of COVID-19 disease, therefore more research with more cases is needed.

CONCLUSION

This comparative study was performed to find out the expression of IL-10 in COVID-19 patients. Traveling and interacting with other infected individuals were the sources of infection for those who contracted the disease, and some were unaware of the cause of the contamination. Different clinical features, such as fever or chills, breathing difficulties, coughing, exhaustion, body aches, pneumonia, loss of taste, and loss of smell, emerged in COVID-19 infectious individuals. Although the p-value was found to be insignificant but high level of IL-10 was observed in the case of IL-10 expression in circulating cells of COVID-19 severe patients as compared to mild symptomatic COVID-19 patients' group and control group. Hence, it is concluded that the proportion of expression of IL-10 was significantly higher in both severe cases of COVID-

19 and mild cases of COVID-19 as compared to controls.

Conflict of interest: The authors declared no conflict of interest.

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Ethical Statement: This project was carried out by with ethical approval number UHS/REG-20/ERC/1758 from the ethical review committee board of university of health sciences.

Ethics Approval: This study was approved by the University of Health Sciences, Lahore, Pakistan. Data Availability All the data related to this study is available with the authors.

Consent Forms: The written consent forms for patients were taken before inducting them into the study. These consent forms are available with the authors.

Authors Contribution: Almina Shafiq and Dr Shahjahan conceptualized the study and wrote the final manuscript. Maria Mustafa and Farah Farooq helped in the analysis and writing the first draft, Mammona Rasool perform the experimental analysis. Ghazal Hussain and Almina Shafiq contributed to manuscript writing & methodology and Dr Shahjahan supervised the whole project.

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