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ALTERATION OF IMMUNOMARKERS AND RELATIVE TELOMERE LENGTH IN COVID-19 PATIENTS

Noor Ul Ain Malik[1], Aftab Ahmad[2], Muhammad Abubakar[1*], Dr Khawar Malik[3], Ayesha Mehboob[4], Nazia Rehman[1], Nasim Akhtar[3], Habib Bokhari[2]

[1]Department of Biosciences, COMSATS University Islamabad, Park Rd, Islamabad Capital Territory 45550, Pakistan

[2]Department of Biosciences, Kohsar University Murree, Murree 47150, Punjab, Pakistan

[3]Pakistan Institute of Medical Sciences, Ibn-e-Sina Rd, G-8/3 G 8/3 G-8, Islamabad Capital Territory 45550, Pakistan

[4]Rawal Institute of Health Sciences, Lethrar Road, Islamabad, Pakistan

E-mails: nourelainmalik@gmail.com AftabahmadKhara@hotmail.com abubakarbbt3@gmail.com drkhawarx@gmail.com ayeshaamehboob@gmail.com Naziarehman@outlook.com drnasimakhtar74@yahoo.com vc@kum.edu.pk

***Corresponding author:** Muhammad Abubakar
*E-mail: abubakarbbt3@gmail.com

Abstract:
Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was first reported in December 2019 in Wuhan, China, and led to a global pandemic. The first case of COVID-19 in Pakistan was reported from Karachi on **February 26, 2020**, since then **1,523,590** cases have been reported with an increasing death toll of 30,340 as of March 2022. Most of the patients showed several mild to moderate symptoms and recovered without the need to be hospitalized. However, a significant number developed severe symptoms like pneumonia leading to acute respiratory distress syndrome (ARDS) with severe medicals complications. The current study investigates the expression of cytokines IL6, IL8, and IL17 in COVID-19 patients with moderate to severe symptoms and patients ventilated due to ARDS concerning in relation to their leukocyte telomere length. 73% of the patients included in this study were male, among them 44% were >60 years. The occurrence of ARDS was observed to be 15.6–31% higher compared to other organ injuries. One of the key factors involved in ARDS is the concomitant increased release of cytokines like IL6, IL8, IL17, etc also known as the cytokine storm, contributing to severity of the disease. Our results show that COVID-19 positive individuals with underlying health conditions and old age showing severe symptoms have increased levels of proinflammatory serum interleukins IL6, IL8, and IL17 by ~6000%, ~2000%, and ~300% respectively. In individuals ventilated due to ARDS, an inverse correlation was seen between critical shortening of leukocyte telomere length and increased levels of interleukins IL6 (*r=-.541, p=0.004*), IL8 (*r=-.235, p=0.009*), and IL17(*r=-.137, p=0.014*). An increase of ~10,000%, ~2,900, and ~600% in comparison with the normal range were seen in IL6,

IL8, and IL17 levels respectively with an enhanced manifestation of disease irrespective of comorbidities and age, suggesting that genetic factors are at play.

Keywords: COVID-19, Immunomarkers, Cytokines, Telomere Length, ARDS

Introduction

At the end of 2019, few cases of pneumonia of unknown etiological pathogenic agents were reported in Wuhan, China. This virus was named "Novel Coronavirus" and WHO officially named this disease Coronavirus disease (COVID-19). When Chinese scientists isolated and rapidly sequenced its genome, the Coronavirus study group (CSG) named it “SARS-CoV-2” (Guo *et al*., 2020). The genomic investigations of this virus showed its 91.02% and 90.55% similarity to Pangolin-CoV and Bat-CoV at the whole-genome level respectively *(T. Zhang et al*., 2020).

In early December, the first case of the virus was observed in one of China’s most populated cities, Wuhan, and began its spread from there. In the city of Bangkok, on January 13th, the first case was reported outside the country (2020) Within 2 months, WHO declared the disease a global pandemic on 11th March 2020, during which 66 countries outside China reported 8568 confirmed cases of the virus, with the highest number reported in Iran and Italy at the time being and 132 confirmed deaths (Hsu *et al*., 2020). Since 11 March, worldwide cases have been on an incline and almost all countries have reported cases.

It is difficult to determine the exact number of individuals infected with the virus, due to the existence of mild symptoms that go unnoticed, however, exponential growth in the number of cases was observed in most low-income countries as reported by World Health Organisation. As of 26 March 2022, over two years since the first reported incidences, over 1.5 million cases have been reported with an increasing death toll of 30,340 in Pakistan (Ministry of National Health Services Regulations & Coordination Pakistan, 2022). Whereas globally, the total cases tally stands at over 476 million. So far COVID-19 has caused a total of 6.1 million deaths. Europe and Americas have the highest number of cases with a total of 347 million and over 4.6 million COVID-19 related deaths (World Health Organization, 2022).

Clinical Manifestation of COVID-19: As COVID-19 primarily resides in the nasal cavity and grows in human airway epithelial cells, hence the signs and symptoms that appear due to this viral infection are mainly related to clinical respiratory syndromes (Perlman & Netland, 2009). Symptoms vary from person to person as some cases are mild, symptomatic while others are asymptomatic and non-pneumonia cases based on immunity (Klompas, 2020; Tian *et al*., 2020). Symptoms that usually appear at the onset of illness are when the virus completes its incubation period which is probably 5 days after the exposure to the virus. Common symptoms that appear in COVID-19 are fever, sore throat, fatigue, tiredness, loss of taste and sense of smell, diarrhea, body aches, hypoxemia, and headache (Ackermann *et al*., 2020; Centre for Disease Control and Prevention, 2020).

It is also responsible for causing progressive life-threatening viral pneumonia. Apart from these most commonly reported symptoms other major changes seen in COVID-affected patients are various endothelial malfunctions e.g., thrombosis, blood pressure & pulmonary embolism (Rothan & Byrareddy, 2020; Sardu *et al*., 2020). Clinical features usually present in chest CT scans include ground-glass opacities, acute respiratory distress syndrome, RNAemia, and acute cardiac injury. The presence of these abnormal characters gradually leads to the death of that patient (She *et al*., 2020).

Cytokine Storm: Acute respiratory distress syndrome or ARDS is a common complication alongside uncontrollable infections (P. Zhou *et al*., 2020). The incidence of reported ARDS was 15.6–31%, higher than that of other organ injuries and roughly 10% of actively infected patients require treatment (Dong *et al*., 2020). About 10% of actively infected patients require treatment for ARDS. While lymphocytes are usually responsible for slowing down diseases’ progression,

increased release of cytokines like IL1B, IL1RA, IL6, IL7, IL8, IL17, etc which is also known as the cytokine storm, is linked with the severity of the disease (Dong *et al*., 2020; She *et al*., 2020).

Interleukin-6 regulates multiple physiological processes such as immune response, hematopoiesis, and inflammation (Tanaka *et al*., 2014) while in the case of viral infections it has also been seen to show pro-inflammatory effects, mediated by the trans-signaling process (Rose-John, 2012). IL-6 levels have been seen to be elevated in three fourth of patients with severe symptoms and a third of patients with minor symptoms of COVID-19 (Wan *et al*., 2020).
Among patients under intensive care, Diao *et al*. found an inversely proportional relation between increased IL-6 levels and T cell counts (Diao *et al*., 2020). Serum levels of IL-6 and CRP have also been reported to be high in a separate study of patients with severe symptoms (X. Chen *et al*., 2020). A study with 452 subjects infected with SARS-CoV-2 shows IL-6 levels were elevated more in patients with harsher symptoms and fatal consequences due to COVID-19 compared to recovered individuals (Qin *et al*., 2020; Y. Zhou *et al*., 2020). In some patients' cytokine storm inclusive of raised levels of IL-6 is associated with cardiac damage (Akhmerov & Marbán, 2020).
Interleukin-8 or neutrophil-activating peptide is a cytokine belonging to the CXC chemokine family (Wolpe & Cerami, 1989). IL-8 is linked to multiple diseases such as viral bronchiolitis, the pathophysiology of the lungs associated with cystic fibrosis, infection, and other inflammatory diseases like rheumatoid arthritis (Srivastava *et al*., 2004). The chemokine interleukin8 (IL8, CXCL8) has a substantial role in inflammatory processes as well as successive wound healing. Elevated Interleukin 6 (IL-6) and Interleukin 8 (IL8) levels were found to be associated with acute stage lung lesions in SARS-CoV-1 patients.

Interleukin-17 produced by Th17 lymphocytes has increased levels in inflammatory conditions and autoimmune diseases (Harrington *et al*., 2005; Duerr *et al*., 2006; McInnes & Schett, 2017). It is a pro-inflammatory cytokine that plays a part in infection, physiological stress, and tissue damage (Isailovic *et al*., 2015; McGeachy *et al*., 2019). Elevated levels of IL-17 have been found in individuals with COVID-19 as part of the cytokine storm (Huang *et al*., 2020) and they have been associated with the viral load and disease severity (Y. Liu *et al*., 2020). Increased IL-17 levels have previously been reported in patients with SARS-CoV and MERS infections (Faure *et al*., 2014; Wu & Yang, 2020).

Genetic Etiology: Genetic makeup of different groups greatly affects their predisposition to viruses through polymorphisms or variants of genes that code for proteins that are abused by SARS-CoV-2, such as Angiotensin-Converting Enzyme 2 (ACE2). It uses ACE2 receptors for attaching to and entering respiratory cells and disrupting the production of proteins that protect cells from the effects of the virus (such as surfactant proteins, but also ACE2 itself) (Guzzi *et al*., 2020). Correlation have been found between COVID-19 susceptibility and ACE2 variants (Benetti *et al*., 2020; Delanghe *et al*., 2020). Other mechanisms of entry may be at play, for example, the transmembrane protease serine 2 gene (TMPRSS2) and its variants have been linked to differences in COVID-19 susceptibility and severity (Asselta *et al*., 2020).
Research has found various polymorphisms in Human Leukocyte Antigen (HLA) to be associated with increased susceptibility to SARS-CoV and MERS-CoV infection. Gene polymorphisms in Mannose-binding lectin (MBL) linked with antigen structure have also been found to be associated with an increased risk of SARS-CoV infection (Li *et al*., 2020). The rs12252-C/C single-nucleotide variant in the Interferon-Induced Transmembrane Protien3 (IFITM3) gene has been proven to be a risk factor for severe influenza infection and has also been seen in COVID-19 patients (Thevarajan *et al*., 2020).
SARS-CoV-2 susceptibility may be mediated by the absence or presence of human histo-blood group antigens (HBGAs) on the surface of gut epithelial cells. The HBGA synthesis is interceded by glycosyltransferases as well as fucosyl-transferases under the genetic influence of the *ABO (H), FUT2* (secretor), and *FUT3* (Lewis), genes. The individuals having an inactivated FUT2 enzyme

also known as non-secretors, do not express blood group antigens and hence be resistant to various viruses. On the contrary, two host proteins members of the oligosaccharyltransferase complex STT3A and STT3B, have been associated with playing roles in the structural formation of vesicles in mammalian cells, and the absence of these proteins has shown a decrease in viral replication (Palm & de Lange, 2008).

Telomere Length: A telomere consists of repetitive nucleotide sequences present at both ends of a chromosome. It protects chromosomal ends from deterioration and from being fused with nearby chromosomes. The sequence of telomeres in vertebrates is 5′-TTAGGG-3′ (J.-P. Liu, 2014) with a reverse complimentary strand and a single-stranded overhang (Witzany, 2008). In humans, this sequence is repeated nearly 2,500 times (Sadava *et al*., 2009) and telomere length (TL) declines from around 11 kb at birth (Okuda et al., 2002) to less than 4 kb in adulthood (Arai *et al*., 2015). The average decline rate is higher in men compared to women (Dalgård *et al*., 2015).

Telomere length is an important biomarker for immune competence. Shorter telomere length is associated with a higher risk of pneumonia as well as hospitalization and death due to infection (Helby *et al*., 2017). Leukocyte telomere length (LTL) shortening is associated with an increase in proinflammatory cytokines synthesis and as well as deteriorated antibody response to vaccines. Shorter LTL is also related to mortality and aging-related morbidity due to conditions that involve the immune system such as cancer and cardiovascular diseases and upper respiratory illness caused by Rhinovirus (Cohen *et al*., 2013). (Fig 1)

Shortening LTL is linked to the process of cell replicative senescence. Cellular senescence may be a key factor that drives predisposition to severe SARS-CoV-2 infection (Malavolta *et al*., 2020). Telomere length may be used to identify the likelihood of COVID-19 infected adult patients dying irrespective of the age group they belong to. According to the experiment carried out by Cohen *et al*, senescence of CD8 Lymphocytes linked with critical TL shortening induces a state of 'hyperfunction' skipping apoptosis, greater secretion of pro-inflammatory cytokines such as tumor necrosis factor-alpha and interleukin-6 as well as the loss of surface CD28, a co-stimulatory receptor necessary to deploy targeted T-cell immune responses (Tsilingiris *et al*., 2020). Therefore, in an acute infection, this cycle may disrupt the effective immune responses and increase the susceptibility to amplified and unrestrained inflammatory responses, exactly like in the case of SARS-CoV-2 'cytokine storm' (Coperchini *et al*., 2020).

Currently, the exact cause of lymphopenia in COVID-19 patients is not properly understood, but rapid immune response recovery necessitates substantial lymphopoiesis, a telomere-dependent phenomenon (Weng *et al*., 1995). The shorter telomeres of hematopoietic cells of the COVID-19 patients belonging to an older age group as well as of men can hinder their lymphopoiesis, particularly lymphopoiesis of CD4/CD8, boosting the possibility of a severe disease outcome. Adults with a shorter TL, regardless of age, could have a predisposition to severe COVID-19-associated drop in CD4/CD8 because their telomeres might be too short to sustain the speedy replicative response of these cells to acute and massive losses of lymphocytes leading to ARDS and even death (Aviv, 2020).

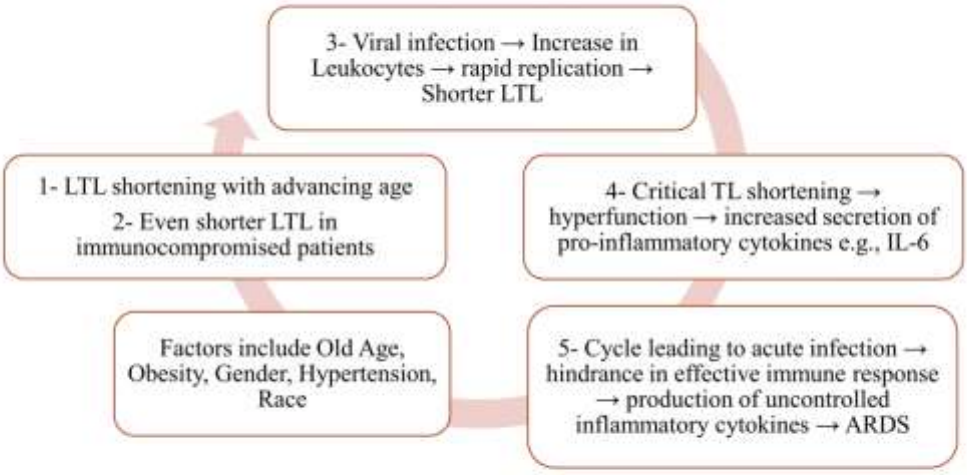

Fig 1: Relationship between short leukocyte telomere length and severity of disease.

Material and Methods

Patients with confirmed COVID-19 positive admitted in the corona Isolation ward of Rawalpindi Institute of Urology & Transplant and Pakistan Institute of Medical Science (PIMS) were selected as the target population. Blood and serum samples were taken from these patients in isolation wards and placed in biohazard bags for safe transfer. A total of 60 samples were collected out of which 14 were critical needing to be ventilated.

DNA Extraction: Genomic DNA was extracted from blood samples (buffy coat) of selected individuals for telomere length analysis with Thermo Scientific GeneJET Genomic DNA Purification Kit (#K0721) using the protocol provided by the manufacturer.

Quantification of DNA: DNA was quantified using nanodrop (IMPLEN, P-330, Germany). (Cuvette sensor and lid factor 10 / 50) The concentration was displayed in ng/µL with A260 / A280 ratio lying between 1.7 – 1.9 determining the purity of DNA being used.

Real-Time Polymerase Chain Reaction: Telomere length measurement was done by real-time PCR (Step 1, Applied Biosystems, USA) using the methodology given by Cawthon (Cawthon, 2002). β-actin house-keeping gene was used as a reference gene. The T/S ratio was calculated using CT values (Nettle *et al*., 2015), which represented telomere length (T) in relation to single-copy gene (S). In the T/S ratio, T represents telomere and S represents the single-copy gene. Primers are listed in Table 1.

Table 1: List of Primers used for amplification of target sequences.

Gene	Primer	Sequence	Annealing Tm	Amplicon Size
β-actin	Forward	TTCTCTGACCTGAGTCTCCTT	56°C	116
	Reverse	ACACCCACAACACTGTCTTAG	56°C	
TEL	Forward	CGGTTTGTTTGGGTTTGGGTTTGGGTT TGGGTTTGGGTT	60°C	39
	Reverse	GGCTTGCCTTACCCTTACCCTTACCCT TACCCTTAACCCT	60°C	

Analysis of IL-6, IL-8, and IL-17 Levels: Interleukins are cytokines secreted by different kinds of cells. Levels of interleukins change in different inflammatory conditions and serve as an important biomarker. Interleukins can be detected in serum by using an Enzyme-linked immunosorbent assay. Kits used for this process were the Elabscience Human IL-6 ELISA kit, Human IL-8 Elisa kit, and Human IL-17 kit. (**Catalog#** E-EL-H0102, E-EL-H6008, E-EL-H0105) following the protocol provided by the manufacturer.

Results

Out of the 60 affected individuals, 44 (73.33%) were males while 16 (26.66%) were female. It was also seen that individuals falling in the 60+ age demographics were the most affected. 28 out of 60 patients were above 60 years of age, making up 44.66% of the subjects under study. Majority of the patients admitted to hospital facilities showed moderate symptoms while a substantial number of patients showed severe clinical manifestations which can be attributed to the early stages of infection and/or underlying diseases.

The most common underlying co-morbidity in COVID-19 affected individuals was seen to be diabetes mellitus (58%), closely followed by individuals with hypertension (43%) that may or may not be present alongside diabetes mellitus. The number of individuals affected with COVID-19 with no underlying conditions was only 23%. Many patients were also affected with cardiac diseases (15%) and a small percentage were affected by other health conditions such as asthma, Parkinson's disease, renal disorders, and cancer.

Analysis of Inflammatory Biomarkers

Interleukin 6: Increased levels of IL-6 were observed in patients falling in the 60+ age demographic as well as in patients with severe to the critical disease condition. Out of these patients, significantly elevated levels of these biomarkers were observed in diabetic and hypertensive patients which suggests that COVID-19 patients with these diseases are at higher risk of uncontrolled hyper inflammation state.

The normal range for IL-6 is 0-10 pg/ml (Ranucci *et al*., 2020) but in patients affected with COVID-19, the level is greatly increased. We observed was seen that patients with critical conditions had abnormally elevated levels compared to patients with moderate severity of symptoms indicating cytokine storm. IL6 levels showed positive correlation with age ($r = 0.518$)

Interleukin 8: Normal range of IL-8 is 6.8–39.65 pg /mL (J. Zhang & Bai, 2017). In patients under study, drastically elevated levels of IL-8 were seen in ventilated patients pointing towards the onset of ARDS and Sepsis. Elevated levels of IL-8 were observed in patients falling in the 50+ age demographic as well as in patients with severe to the critical disease condition. Significantly increased levels of these biomarkers were observed in diabetic and hypertensive patients indicating increased inflammation levels. IL8 levels showed positive correlation with age ($r = 0.342$).

Interleukin 17: IL17 is a key factor in a cytokine storm, causing alveolar inflammation and poor prognosis in ARDS. An increase in IL17 levels leads to an increase in IL-17 regulated cytokines like IL6 and IL8. Normal range of IL-17 is < 27 pg/mL (Jaszczura *et al*., 2019). Results suggest that IL17 levels are directly proportional with disease severity and lung injury as ventilated patients demonstrate extremely elevated levels of IL17 compared to patients showing moderate to severe symptoms. A positive correlation was seen between IL-17 levels and age ($r = 0.139$)

Telomere Length Analysis

Telomere length shortening has been found to have a negative correlation with age progression (López-Otín *et al*., 2013). Telomere length naturally shortens with age. A significantly negative correlation was seen between age and telomere length shortening in COVID-19 patients. (Table 2)

Table 2: Correlation between age and Relative Telomere Length (RTL)

		RTL	Age
RTL	Pearson Correlation	1	-.712**
	Sig. (1-tailed)		.003
	N	60	60
Age	Pearson Correlation	-.712**	1
	Sig. (1-tailed)	.003	
	N	60	60
**. Correlation is significant at the 0.01 level (1-tailed).			

In a comparison between relative telomere lengths (RTL) in COVID-19 patients across different age groups, age groups 41 & above showed a rapid decline in RTL. In a comparison between Average T/S ratios and Average RTL in controls and COVID-19 patients across different age groups, the shortest RTL was observed in the age group 61+ which can mainly be attributed to age. RTL of controls versus patients with moderate symptoms and ventilated patients across different age groups showed that while patients falling in the 51+ age demographics show the shortest relative telomere length, ventilated patients show the shortest RTL, which can, in part be attributed to ARDS.

Patients were divided into 3 groups of Only COVID-19, COVID-19, and diabetes and COVID-19 compounded with other diseases which include HTN, Asthma, Parkinson's disease, tuberculosis, and Cancer. In age groups, 31 to 50, patients with COVID-19 compounded with other diseases showed the shortest RTL. In age group 51+, Diabetic patients with COVID-19 showed the shortest RTL. It is noteworthy that in 61+ demographics, patients with only COVID-19, of which most were ventilated due to ARDS showed the shortest RTL, pointing towards the possibility that TL shortening due to age compounded with viral infection leads to critical TL shortening and hence ARDS. (Fig 2)

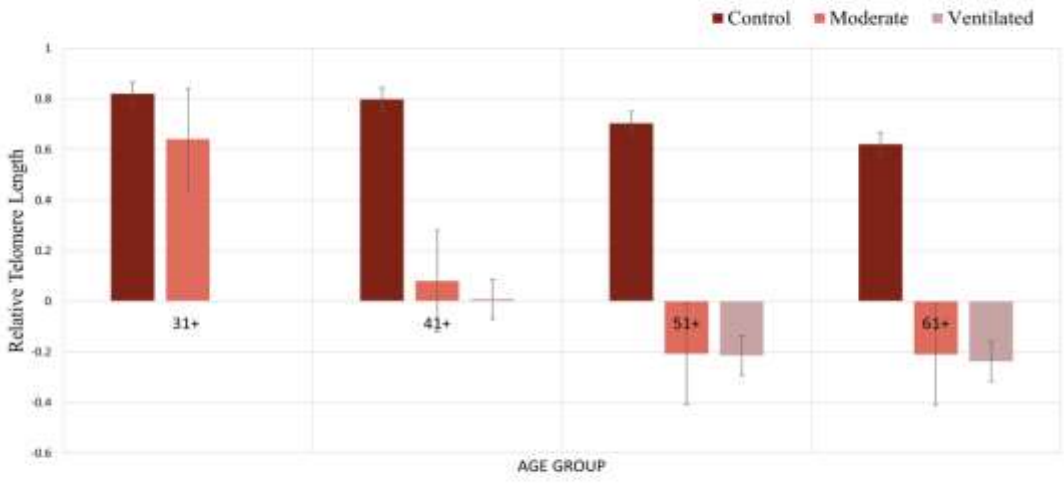

Fig 2: Relationship between leukocyte telomere length, COVID-19 severity and age

Relative Telomere Length and Interleukins: Relative Telomere Length showed a significant negative correlation with IL6 suggesting that TL decreased with rapid increase in IL6 levels. (r = -.54, p = 0.004) (Table 4) RTL showed a negative correlation of weak significance with IL8. (r = -.235, p = 0.009) (Table 5) IL17 showed a negative correlation of negligible significance with RTL in COVID-19 patients. (r = -.137, p = 0.14) (Table 6)

Table 3: Correlation between IL6 and Relative Telomere Length

		RTL	IL6
RTL	Pearson Correlation	1	-.541**
	Sig. (1-tailed)		.004
	N	60	60
IL6	Pearson Correlation	-.541**	1
	Sig. (1-tailed)	.004	
	N	60	60
**. Correlation is significant at the 0.01 level (1-tailed).			

Table 4: Correlation between RTL and IL18

		RTL	IL8
RTL	Pearson Correlation	1	-.235
	Sig. (1-tailed)		.009
	N	60	60
IL8	Pearson Correlation	-.235	1
	Sig. (1-tailed)	.009	
	N	60	60

Table 5: Correlation between Relative Telomere Length and IL17

		RTL	IL17
RTL	Pearson Correlation	1	-.137
	Sig. (1-tailed)		.014
	N	60	60
IL17	Pearson Correlation	-.137	1
	Sig. (1-tailed)	.014	
	N	60	60

Discussion & Conclusions:

This study aimed to analyze levels of proinflammatory cytokines IL-6, IL-8, and IL-17 in COVID-19 positive patients and their relative telomere length. A total of 60 samples were collected from the Pakistan Institute of Medical Science and Rawalpindi Institute of Urology and Transplant during the first peak of COVID-19 between June and October 2020. Patients were divided into three groups i.e., Mild, Moderate to Severe, and Critical/Ventilated based on their condition at the time of sample collection. Keeping in view the goal of the study, patients with mild symptoms were excluded from further analysis.

The rate of mortality and poor prognosis in COVID-positive patients with severe infection is very high even after hospitalization (Jose & Manuel, 2020). Majority of these hospitalized patients with a severe infection have complications such as pneumonia, pulmonary edema, damage to airways, and ARDS which is responsible for 70% of deaths in these complicated cases (Huang *et al*., 2020b; Wang *et al*., 2020). There is substantial evidence that COVID-19 severity is associated with an increase in levels of proinflammatory cytokines and chemokines such as IL6, IL8, and IL-17 (Hojyo *et al*., 2020).

IL6 is one of the most important biomarkers in COVID-19 as the increase in its level indicates viral infection or internal injury. Elevated IL6 levels have been observed in COVID-19 positive patients with a mean increase of three times or more compared to the normal range in patients with complicated infections (Aziz *et al*., 2020). Our results show a drastic increase in IL6 levels in ventilated patients and patients with underlying conditions such as diabetes and hypertension. Results also indicate a positive correlation between the increase in IL6 levels and age.

IL8 is a pro-inflammatory chemokine that is involved in the release and activation of neutrophils in inflammation and injury. Chen *et al* reported that patients with high levels of IL-8 showed a higher probability of admission to ICU and mortality (L. Chen *et al*., 2020). We found an inclining trend in

IL-8 levels with an increase in severity of infection, with the highest levels of IL-8 seen in patients ventilated due to ARDS. IL-8 levels also showed a positive correlation with an increase in age.
IL17 is a key player in cytokine storm in ARDS caused not only by COVID-19 but in previous widespread infections caused by viruses such as MERS and H1N1 (Mikacenic *et al*., 2016). Various studies have shown a positive correlation between levels of IL17 and lung injury in severe cases of COVID-19 (Y. Liu *et al*., 2020). IL17 is responsible for the early recruitment of neutrophils into the lungs, causing severity and poor prognosis (Wu & Yang, 2020). With an increase in IL-17 levels in complicated cases, levels of IL-17 regulated cytokines such as IL6 and IL8 also increase in a cascading manner, marking the severity of infection (X. Chen *et al*., 2020). (Table 3)

Table 6: Correlation between IL6, IL8, and IL17

		IL6	IL8	IL17
IL6	Pearson Correlation	1	.247	.735**
	Sig. (1-tailed)		.055	.000
	N	60	60	60
IL8	Pearson Correlation	.247	1	.285*
	Sig. (1-tailed)	.055		.032
	N	60	60	60
IL17	Pearson Correlation	.735**	.285*	1
	Sig. (1-tailed)	.000	.032	
	N	60	60	60
**. Correlation is significant at the 0.01 level (1-tailed). *. Correlation is significant at the 0.05 level (1-tailed).				

We found a direct correlation between IL17 levels and disease severity, with the highest concentration in ventilated patients with ARDS. A significant increase in Il-17 levels was observed in older age demographics.
An interesting finding of the study was that about 50% of the ventilated patients with a critically severe infection had no underlying conditions to complicate their case. Of the 15 ventilated patients, only 6 were females. 8 patients had no other disease besides COVID-19 and out of these 8 patients, 5 belonged to the under 50 age demographics, indicating the contribution of genetic factors towards the severity of the disease.
Telomere length is one of these genetic factors that greatly increase susceptibility towards infection and weak immune response. Individuals with shorter LTL (leukocyte telomere length) have a greater predisposition to infection-related hospital admission and mortality (Helby *et al*., 2017). Also, critical TL shortening induces a state of 'hyperfunction' resulting in an increased secretion of pro-inflammatory cytokines such as tumor necrosis factor-alpha and interleukin-6 leading to a severer infection (Cohen *et al*., 2013). As reported by Barrett and Richardson, males have a shorter telomere length and a higher attrition rate that may be one of the reasons for high mortality rates in men due to COVID-19 (Barrett & Richardson, 2011).
Our results show that COVID-19 positive individuals with underlying health conditions and old ag showing severe symptoms have increased levels of proinflammatory serum interleukins IL6, IL8, and IL17 by ~6000%, ~2000%, and ~300% respectively. However, individuals ventilated due to ARDS show double the increase in levels of interleukins IL6, IL8, and IL17 by ~10,000%, ~2,900, and ~600% respectively with an enhanced manifestation of disease irrespective of comorbidities and age suggesting that critical telomere length shortening is indeed a major contributing factor in ARDS due to COVID-19.

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Dr. Aneela Javed, Atta Ur Rehman School of Biosciences, National University of Science and Technology, Islamabad, Pakistan

Dr. Ramla Shahid, Department of Bioscience, COMSATS University Islamabad, Islamabad, Pakistan

Author Contribution:
Noor Ul Ain Malik – Literature Review, Sampling, Experiment Design, Experimental Work, Result Analyses.
Aftab Ahmad – Experiment Design, Methodology, Resources
Muhammad Abubakar – Experiment Design, Methodology,
Dr. Khawar Malik - Sampling, Methodology, Resources
Ayesha Mehboob – Primer design, Methodology
Nazia Rehman – Primer design, Methodology
Dr. Nasim Akhtar – Sampling, Methodology, Resources
Habib Bokhari – Principal investigator, Experiment Design

Conflict of interest statement:
The author declares that they have no conflict of interests.

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