Assessment of therapeutic response of the Tocilizumab in SARS-CoV-2 associated cytokine storm: A single center experience

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Abstract

Background: Critical COVID-19 patients may present with a cytokine storm-like phenomenon. IL-6 is found to be a key player in this cytokine storm, and COVID-19 severity is directly linked to the IL-6 levels.
Objective: To assess the response of Tocilizumab during the inflammatory phase of COVID-19 patients.
Methodology: This cross-sectional study was based on a retrospective collection of data from the records of a total of 17 COVID patients, admitted to the COVID Unit of Farooq Hospital, Lahore, from 1st October 2020 to 30th December 2020. The ethical review board of Akhtar Saeed Medical and Dental College, Lahore approved the research. All these patients received 8-800 mg/kg of body weight Tocilizumab intravenously for pneumonia in COVID-19 patients. All these patients were evaluated for IL-6 levels in serum initially for 2-3 days (24-48 hours) before and after the 3rd day and 7th day of Tocilizumab infusion. The Electrochemiluminescence immunoassay method was used to quantify serum IL-6. The SPSS (v. 22.0) was used for analysis.
Results: The analysis shows that the median (IQR) IL-6 levels prior to treatment with Actemra, three days after Actemra administration, and seven days after Actemra was administered, were 1879 (IQR=726-2842), 1136 (IQR=499-2266), and 438 (IQR=151-1085), respectively. The change in Il-6 levels before and after Actemra administration was statistically significant, (p=0.001). A notable difference was also observed between IL-6 levels with respect to Actemra administration after three and seven days (p=0.04). There was a statistically significant median difference in IL-6 levels seven days post-Actemra in patients who survived and died, (*P*=0.01).
Conclusion: Tocilizumab administration along with sequential laboratory investigations particularly IL6 might be helpful in reducing the complications of cytokine storm in patients with severe COVID-19 pneumonia, resulting in better outcomes.

Keywords: Cytokine release syndrome (CRS), Interleukin-6 (IL-6), Tocilizumab (TCZ), Critically Ill.
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Introduction

The COVID-19 pandemic has changed the world. The global death toll and healthcare burden have skyrocketed. COVID-19's clinical presentations vary from mild to severe ones.[1] The intensity of COVID-19 is assessed by cytokine release syndrome (CRS), in which numerous cytokines are generated as a result of an inflammatory cascade.[2,3] In the cascade stage, victims suffer from grave symptoms, ranging from heart damage to malfunctioning of multiple organs, and even death.[4] Any intervention, which leads to immediate diagnosis and early treatment of cytokine storm can prove to be life-saving.

Interleukin-6 (IL-6) plays an integral part in immune and inflammatory response.[5] literature has shown that previous studies have highlighted the major role of IL-6 in cytokine release syndrome (CRS).[5,6] Among the impacts of COVID-19 in patients is a response similar to CRS. The flow cytometry of COVID-19 has identified a rise in Helper T cells, which are triggered by pro-inflammatory cytokines including IL-6. This leads to immunity in the course of infection.[7] COVID-19 patients have been observed to have upregulated pro-inflammatory cytokines. A recent study highlighted an increased amount of pro-inflammatory cytokines, as IL-6 levels were increased in severe cases as compared to milder ones, thus showing the link between the severity of COVID-19 and cytokine storm.[8] Therefore, early interventions can save patients from ending up in cytokine storms.[7,9]

COVID-19 pneumonia patients had raised ESR, CRP, IL-6, and ferroprotein.[10] Inflammatory cytokine storm in moribund COVID-19 patients is related to elevated plasma IL-6. Several recently conducted clinical studies on COVID -19 patients have shown higher levels of IL-6 in critical patients, thus for severity evaluation, IL-6 can be used as a

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biomarker.[11-13] Patients with severe cytokine storm show higher levels of inflammatory cytokines, than those with milder forms of COVID-19.[14]

Among dead patients, serum levels of interleukin 10 (IL-10), interleukin 2 receptor (IL-2R), interleukin 8 (IL-8), and tumor necrosis factor α (TNFα) and IL-6 were considerably greater compared to recovered counterparts.[15] The serum levels of cytokines were lower in milder cases than in severe ones.[16] Several pharmaceutical drugs having antiviral properties or their ability to manage the hyper-inflammatory stage have been offered as COVID-19 therapy possibilities. ClinicalTrials.gov lists over 400 clinical trials that are recruiting to investigate numerous possible therapies. However, there is currently insufficient data to justify the usage of these drugs.[17] To address the hyper-inflammatory phase of COVID-19, clinicians are using IL-6 antagonists to curb CRS. The researchers are looking into the use of IL-6 inhibitors for COVID-19 treatment. These drugs are tocilizumab, sarilumab and siltuximab.[5] Tocilizumab, a monoclonal antibody against IL-6 receptor, is a drug of choice. Tocilizumab binds to the membrane-bound and soluble IL6 receptors will lead to the initiation of IL6 biological activity.[6,18] It may prove beneficial in COVID-19 infected patients with elevated IL-6 levels.

The COVID-19 pandemic has had a significant impact on the world economy and the healthcare system. While awaiting the development of an effective vaccine, finding treatments for COVID-19 patients in serious or critical conditions is vital for minimizing mortality and easing the burden on the healthcare system. One possible treatment may be based on inhibiting CRS with anti- IL-6 agents to help to alleviate the symptoms, decrease hospital stays and lessen the requirement for oxygen treatment, and eventually better outcomes. So far convincing data has not yet been generated for double-blind studies, but even then the IL-6 receptor inhibitors, such as tocilizumab (TCZ) is added in the clinical guidelines for severe COVID-19 therapy.[19] The current study has emphasized the role of TCZ in the treatment of cytokine storms and has focussed on the inclusion of TCZ in the management regime for COVID-19 patients. The purpose of this study was to see if tocilizumab may help individuals with COVID-19 infections, especially those who were in the inflammatory stage of the disease.

Methodology

This was a cross-sectional study based on the retrospective collection of the data of a total of 17 COVID patients admitted in the COVID Unit of Farooq Hospital, West Wood, Lahore between 1st October 2020 to 30th December 2020. The research was authorized by the Research Ethical Committee (IRB) of Akhtar Saeed Medical and Dental College, Lahore, and formal approval was taken from Medical Superintendent Farooq Hospital, West Wood, Lahore (FHWW-IRB/CU/08/3-2020). All these patients received 8-800 mg/kg of body weight tocilizumab (Brand name; Actemra) intravenously for COVID-19 pneumonia. All of them were evaluated for serum IL-6, 24 to 48 hours before and after the 3rd day and 7th day of tocilizumab infusion. The Electrochemiluminescence immunoassay method was used to quantify serum IL-6.

The SPSS (v. 22.0) was used to analyze the data. The assumption of normality in IL-6 levels at three-time intervals was seen through the Shapiro-Wilk test. As the *p*-values were less than 0.05 in all three-time intervals, non-parametric statistical tests were used. To see whether the intervention of Actemra was successful or not in decreasing Interleukin-6 (IL-6) levels, the Freidman ANOVA test was used. Additionally, analysis with Wilcoxon signed-rank test was conducted for pair-wise comparisons of the three time intervals. The results were summarized in the form of a line graph for better visual presentation. The median IL-6 levels at different time intervals in COVID-19 patients who survived or died were compared using the Independent Samples Mann-Whitney U Test. To assess median differences with respect to various clinical characteristics in COVID-19 patients at the time of admission and discharge, Wilcoxon signed-rank test was conducted.

Results

The analysis shows that the median (IQR) IL-6 levels prior to treatment with Tocilizumab (Brand name: Actemra), three days after Actemra administration, and seven days after Actemra was administered, were 1879 (IQR=726-2842), 1136 (IQR=499-2266), and 438 (IQR=151-1085), respectively. There was a statistically significant difference in IL-6 levels before and after Actemra

was administered, $\chi2(2) = 23.06$, P=0.001. There was a considerable difference in the pair-wise comparisons between IL-6 levels before Actemra was administered and three days post-Actemra administration (Z = 2.40, P= 0.04). Likewise, there was a statistically significant difference between IL-6 levels before Actemra was administered and seven days post-Actemra administration (Z = 4.80, P=0.001). There was also a statistically significant difference between IL-6 levels with respect to Actemra administration after three and seven days (Z = 2.40, P= 0.04).

The comparison of IL-6 levels in COVID-19 individuals who survived or died revealed no statistically significant median variations of IL-6 levels before and three days post-Actemra administration, respectively (Table-I). There was a statistically significant median difference in IL-6 levels seven days post-Actemra in patients who survived and died, $\chi2(2) = 2.39$, P<0.05. The descriptive statistics suggest (see Table-I) that there was a higher median reduction of IL-6 levels in patients who survived (1824 to 281) whereas the median levels of IL-6 slightly increased in patients who died (2785 to 2833).

The comparison of clinical characteristics in patients with COVID-19 at the time of admission and discharge is presented in Table II. The results show that the median difference in hemoglobin in patients at the time of admission and discharge was statistically significant, [$\chi2(2) = -2.74$, P<0.01]. Likewise, the median differences with respect to protein [$\chi2(2) = -3.39$, P<0.01], albumin [$\chi2(2) = -2.59$, P<0.05], and urea [$\chi2(2) = 2.06$, P<0.05] levels at the time of admission and discharge were also significant. Moreover, there was also a notable median difference in CRP level at the time of admission and discharge, [$\chi2(2) = -3.20$, P<0.01]. The median differences in remaining characteristics at the time of admission and discharge were not statistically significant (Table-II).

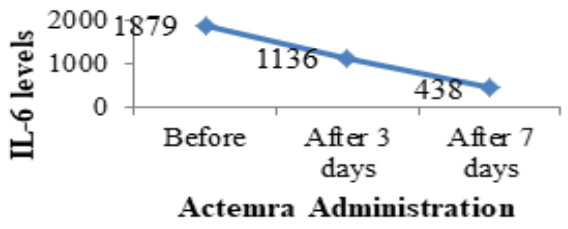

Figure-I: Median IL-6 levels before and after Actemra administration in COVID-19 patients

Table-I: Comparison of Median IL-6 levels in COVID-19 patients who survived or died

IL-6	Survived (n = 14)	Died (n = 3)	*P*-value
Before (TCZ) Actemra	1824	2785	0.244
3 days (TCZ) Post-Actemra	722	2806	0.091
7 days (TCZ) Post-Actemra	281	2833	0.012

Table-II: Comparison of Laboratory Profiles in Coronavirus disease 2019 (COVID-19) patients at the time of admission and discharge

Laboratory Profiles (Units)	Admission	Discharge	*P*-value
Hemoglobin (g/dL)	13.6 (12.5 – 14.4)	12.6 (11.5 – 13.1)	***0.006***
Total Leucocyte Count (x10^9/L)	10.2 (7.2 – 11.4)	10.8 (9.5 – 16.2)	0.072
Platelets (x10^9/L)	178 (156.5 – 270)	178 (140 – 251)	0.570
Neutrophils (%)	80 (77 – 87)	86 (79.5 – 91.5)	0.265
Absolute Neutrophils (x 10^9 /L)	7.96 (5.65 – 9.63)	9.50 (7.70 – 13.9)	0.062
Absolute Lymphocytes (x 10^9 /µL)	1.15 (0.64 – 1.63)	0.97 (0.74 – 1.48)	0.943
Lymphocytes (%)	12 (8.5 – 18)	10 (5.5 – 16)	0.275
Bilirubin (mg/dL)	0.70 (0.60 – 1)	1 (0.75 – 1.30)	0.155
Aspartate Amino-transferase (U/L)	53 (36 – 78.5)	36 (30 – 69.5)	0.485
Alanine Aminotransferase (U/L)	60 (31.5 – 90.5)	58 (39.5 – 114)	0.266
Protein (g/dL)	6.20 (6 – 6.45)	5 (4.85 – 5.85)	***0.001***
Albumin (g/dL)	3.50 (3.25 – 3.75)	3.20 (2.75 – 3.45)	***0.010***
Urea (mg/dl)	44 (39.5 – 53.5)	68 (42 – 145.5)	***0.039***
Creatinine (mg/dl)	1.10 (0.90 – 1.30)	120 (1.05 – 2.70)	0.138
Sodium (mmol/L)	136 (130 – 138)	136 (134.5 – 141)	0.209
Potassium (mmol/L)	4 (3.60 – 4.30)	3.90 (3.55 – 4.70)	0.460
C-Reactive Protein (mg/L)	68.1 (49.3 – 81)	3.40 (1.50 – 6.70)	***0.001***
D-Dimer (µg/mL)	0.56 (0.37 – 0.97)	0.53 (0.37 – 5.40)	0.795

Results are shown as Median and Interquartile Range (between brackets)

Discussion

In this research, the effectiveness of TCZ (Actemra) in the management of COVID-19 patients who were extremely ill was investigated at regular intervals before TCZ administration, 3rd and 7th days after hospital admission. The administration of TCZ could interfere with disease progression rates and thus ameliorate COVID-19-related symptoms.

In the current study, the median (IQR) IL-6 levels prior to Actemra treatment, then three and seven days after Actemra was administered, were 1879 (IQR=726-2842),1136 (IQR=499-2266), and 438 (IQR=151-1085), respectively. Following the delivery of Actemra, there was a declining trend in IL-6 levels. Similar findings were reported by Luo et al[20], whose study showed the promising role of TCZ in managing IL-6 related cytokine storms in COVID-19 victims with serious conditions. In Spanish research, reference point IL-6 levels of COVID-19 critically ill participants had been determined before TCZ administration and then 3rd day and 9th-day post-TCZ administration. A significant drop in IL-6 levels was observed after TCZ infusion. Higher IL-6 levels at baseline predicted a high risk of shock and mortality, however, timely administration of TCZ significantly improved life expectancy in critically ill patients.[21] Another recently published Indian study supported the utilization of TCZ in critically unwell COVID-19 patients. Being an IL-6R inhibitor, the TCZ lowers previously raised serum IL-6 levels when recorded on day 3 post-TCZ infusion, in severely ill COVID-19 individuals.[22] COVID-19 infection in renal transplant patients had a favorable clinical response to TCZ as observed by Gautier-Vargas et al, as within 2 days of receiving TCZ, blood IL-6 levels dropped from 430.8 pg/mL to 3.4 pg/mL).[23]

All these findings of the current research are consistent with the aforementioned investigations. In the current study, IL-6 levels were dropped from 1824 (pre-TCZ) to 281 post-TCZ administration. However, those who couldn't survive had initially very high levels of IL-6 (2785) that kept on increasing till they die (2833) despite TCZ infusion. In a Spanish study, after taking relevant clinical details and baseline laboratory findings including serum IL-6 levels, severe COVID-19 victims underwent TCZ therapy. Post-TCZ, the clinical information and laboratory findings were repeated on the 3rd and 9th days for comparative analysis. IL-6 levels were markedly reduced after TCZ administration which led to gradual improvement in COVID-19 patients.[24] In the current investigation, conducted on COVID-19 patients, initially TCZ was administered within the first 24 hrs, and then followed by a steroid (methylprednisolone). Several laboratory parameters including IL-6 were recorded at regular intervals (days 0, 3, and 6) of therapy. The median IL-6 levels prior to the TCZ (342.50 pg/mL) were found to be significantly elevated when compared with post-TCZ IL-6 levels on the 3rd day (563 pg/mL, *P*=0.00001). However, median IL-6 levels were then reduced to 545pg/mL on the 6th day of TCZ administration. The initial rise in IL-6 levels has been attributed to a cytokine storm by many researchers.[24]

A retrospective study was conducted on 43 COVID-19 patients, receiving TCZ at the American Hospital and Koc University Hospital in Istanbul, Turkey. The laboratory investigations were carried out two weeks post-TCZ, for comparative analysis in severe and critically ill COVID-19 patients. A statistically significant difference in IL-6 levels between severe and critical patients was observed (115 & 168 respectively, $P = 0.025$).[25] In a meta-analysis of studies on IL-6 levels and clinical outcomes in patients suffering from COVID-19, the mean IL-6 levels in complicated patients were significantly elevated as compared to non-complicated cases. The elevated IL-6 was correlated with the death rate. Out of these large number of studies, 5 reflected a correlation between IL-6 and mortality in COVID-19 patients. It was inferred that the higher the levels of IL-6 on admission, the greater the chances of mortality.[26] Similarly, patients with IL-6 levels of more than 30 pg/mL reflect severity index and would be requiring invasive mechanical ventilation in later stages.[16]

In the current study, post-TCZ Hb levels dropped down from a mean of 13.6 (on admission) to 12.6 (on discharge) with a significant *P*-value of 0.006. The comparative laboratory values (at the time of admission and discharge) were recorded as TLC (10.2 & 10.8), platelets (178 & 178), neutrophils (80 & 86), absolute neutrophils (7.96 & 9.50) and absolute lymphocytes (1.15 & 0.97) and

lymphocytes (12 & 10). All these values were within the normal range. In an Indian study, the laboratory findings of 30 extremely or critically unwell COVID-19 patients were recorded serially, initially before TCZ infusion and then at regular intervals on days 1, 3, 5 & 7. The mean values for Hb, TLCs, and platelet counts were within the normal range with mean hemoglobin of 13.60 gm/dL, TLCs 7. 2 7/ mm^3, and platelet counts were 2.40 Lac/μL. However, significant fluctuation within serum IL-6 levels was observed, quite raised initially then the levels were dropped down gradually in the following days (mean IL-6= 298.4 pg/mL, 305.9 pg/mL, 180.9 pg/mL, and 84.5 pg/mL at days 1, 3, 5 & 7 respectively ($t = 13.33$, $P<0.001$).[22]

All patients had lymphopenia (less than 1×10^3cells/uL) at the time of TCZ treatment. On the contrary, there were also elevated amounts of markers of inflammation such as CRP, fibrinogen, ferritin, and IL-6. The lymphocytes number rose particularly in the healthier participants. CRP, fibrinogen, and ferritin levels returned to normal levels. Whereas, D-dimer and Il-6 levels were higher equally in better and deteriorated patients.[27]

In a case series of COVID-19 infected individuals who received TCZ therapy, 92% of the patients (n=35) had low amounts of lymphocytes prior to TCZ delivery. However, unlike other research,[28] the lymphocyte count of only 3 patients was restored to the normal limit (20%-51%) within 7 days of receiving TCZ. Conversely, in another study done on a group of severely critically ill patients, the levels of CRP and D-dimer were remarkably lower in severe cases in contrast to critically ill patients ($P = 0.0029$ and $P = 0.002$, respectively). The lymphocyte percentage was better (13.5%) in severe cases as compared to the critical ones (11%, $P = 0.007$).[25] In a randomized control trial, CRP and neutrophil levels rapidly declined in the TCZ administered patients, and lymphocyte count was increased. The CRP levels got stabilized after day 4 of TCZ administration.[29]

In a study conducted on 112 patients from El Paso, Texas, CRP levels were initially over the normal in a majority of patients before TCZ treatment but declined dramatically with TCZ in the days following. The median CRP readings showed declining levels from 11 (6-18.75) mg/L on days 0 to 5 (2-13) and 2 (1-4) mg/L on days 3 and 6, respectively (P=0.00001), as compared to pre-TCZ therapy.[24] A significant mean difference of CRP (8.4 mg/dl; P<0.001) was reported in a study from Peshawar, Pakistan before TCZ and after administering TCZ.[30] There was a case series study done on 38 COVID-19 patients, in a community teaching hospital located in Brooklyn, New York. The inflammatory serum markers were assessed at baseline, on days 4 and 7 after TCZ therapy, and at discharge from the hospital (or death). Although CRP, LDH, and ferritin all dropped after TCZ treatment, only CRP showed a significant change. The median CRP levels reduced considerably from 189.9 mg/L to 54.8 mg/L (P =0.003), 72 hours after TCZ delivery.[31]

Luo et.al from Wuhan, China reported a decline in CRP levels from 119 to 113 mg/dl in patients who received TCZ medication. Following TCZ, D-dimer levels fell from 12.5 g/ml to 10.3 g/ml (P=0.643), according to our observations.[20] The difference in D-dimer pre-TCZ and post-TCZ treatment was not significant, however, a sharp decline was observed between day 3 and day 6 after initiating TCZ. D-dimer levels were 1.2 (0.66-1.84) mcg/mL on day 6, compared to 1.2 (0.92-2.05) mcg/mL on day 3 (P =0.03).[24] Toniati et al. from Italy, on the other hand, recorded an increase in D-dimer levels.[27] In this study, bilirubin, AST, ALT, and albumin levels on hospitalization and discharge were recorded (0.70 & 1, 53 & 36, 60 & 58, and 3.50 & 3.20) only albumin was significant among these readings.

In a recently published Iraqi study, the liver enzyme tests were assessed in the serum of COVID-19 patients. Of the 74 patients, 25 (34.7%) and 28 (40%) showed aberrant ALT and AST activity respectively, whereas 39 (52.7%) had remarkably abnormal total bilirubin ($P < 0.05$).[32] In a study, the severity of covid-19 illness is shown by increased AST and ALT values and decreased albumin levels.[33] Similarly Guan *et al.*[1] also found that severe group participants had elevated AST (39.4% vs. 18.2%) and ALT (28.1% and 19.8%, respectively) than non-severe group individuals. Additionally, a Chinese study found the raised levels of ALT and AST in ICU admitted patients (P=0.007 and P=0.001).[34] Another study from China reported that the severe patient group exhibited considerably elevated ALT (P=0.015), and AST ($P < 0.001$) levels, but lower albumin levels ($P < 0.001$) than the non-severe patient's group. When compared to non-severe COVID-19 patients, severe patients had higher rates of abnormal AST and albumin ($P < 0.001$ in both).[35] A meta-analysis regarding COVID-19 patients concluded that more than 80% of patients presenting with liver

malfunctioning had lower albumin levels, which had a clear impact on overall prognosis and clinical outcome in these patients.[36] All these studies indicate that liver injury contributes to disease severity course in COVID-19 patients.

Nonetheless, it appears for severely ill patients or patients with extraordinarily high IL-6 levels, repeating the dose every day, every other day, or every three days with a total of two to three doses would be reasonable. Owing to prolonged half-life and saturate qualities of receptor binding, the dose of TCZ could be lowered with recurrent administration.[20]

The current study had certain limitations as this was a retrospective descriptive study design that had only a small sample size. There was no control group, and it was a single-center based study. In order to validate the results, large-scale studies are suggested.

Conclusion

Tocilizumab administration along with sequential laboratory investigations particularly IL-6 might be helpful in reducing the complications of cytokine storm in patients with severe COVID-19 pneumonia. The timely administration in critically ill patients had better outcomes as compared to others without Tocilizumab administration.

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