**ORIGINAL ARTICLE**

# Analysis of Serial C-Reactive Protein Levels in Critically Ill COVID-19 Patients Receiving Tocilizumab

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**ABSTRACT**

***Background and Objective*:** COVID-19 can cause severe acute respiratory distress syndrome. With deteriorating disease, most of the patients may require intensive care admission. This study was carried out to determine and evaluate the response of Tocilizumab with special reference to C-reactive protein (CRP) in critically ill patients presented to Farooq Hospital, West Wood Lahore.

***Methods***: This retrospective study included the data of 55 critically ill COVID-19 patients (respiratory rate ≥30, $SpO_2$<93%, oxygen requirement ≥5L/min, $PaO_2/FiO_2$ ≤300 mmHg) admitted in Corona unit of Farooq Hospital West Wood Lahore, who were being treated with Tocilizumab alongwith standard treatment protocol between April 27 and June 21, 2020. The data has been retrieved from hospital records after taking appropriate permission and consent. Demographic, clinical features and serum CRP were recorded for each of them, before and after administration of Tocilizumab. Data analysis was done by Statistical Package for the Social Sciences (SPSS) version 22.0 and expressed as frequency and percentages.

***Results:*** Out of 55 patients who were administered Tocilizumab, 72.7% survived whereas 27.3% died. There was higher median reduction of CRP levels in patients who survived (77.5 to 34.9 mg/L) as compared to those who died (65.5 to 45.3 mg/L). There was a statistically significant difference between CRP levels at the time of admission, 72 hours after Tocilizumab was administered (P<0.0001).

***Conclusion:*** Tocilizumab administration might be helpful in reducing the complications of cytokine storm in patients with severe COVID-19 pneumonia.

**KEYWORDS:** C-reactive protein, Pneumonia, COVID-19, Tocilizumab, Critically ill.

***How to Cite This:***

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## INTRODUCTION

COVID-19 can cause severe acute respiratory distress syndrome. With deteriorating disease, most of the patients may require intensive care admission. According to recent statistics, the mortality rate in critical patients may exceed 60%.[1]

A number of studies have shown that a vast majority of asymptomatic patients may present with abnormal findings on imaging studies. Hence the role of serum biomarkers in clinical outcome of COVID-19 disease remains indispensable. Among these serum biomarkers, C-reactive protein (CRP) has gained much attention because its levels in

blood correlate significantly with the disease progression. Raised levels of CRP indicate active inflammation and infection. Under normal circumstances, the serum CRP levels remains below 5 mg/L. With progressing infection, the concentration of CRP increases rapidly and reaches to its maximum within initial 48 hours of disease onset. After the halt of inflammatory process, the CRP level drops down to optimum concentration. CRP is specifically involved in activation of complement pathway of immune system and phagocytosis to fight against pathogens.[2] Similarly the levels of CRP and ferritin are observed to be raised in patients suffering from severe COVID-19 disease.[3]

The severity index of COVID-19 is attributed to raised CRP levels in blood, a fundamental marker of COVID-19 disease, which can highlight the chances for morbidity and mortality.[4]

The detailed pathogenesis is still not known. However, this is well established fact that COVID-19 infection causes a cytokine storm, releasing substantial amounts of proinflammatory cytokines (IL-6, TNF alpha, 1L-12). In a study conducted on autopsy of a patient who died from severe COVID-19, biopsy samples from lungs revealed bilateral alveolar damage and mononuclear lymphocytic infiltrates.[5]

The cytokine storm can have deleterious effects on patients overall clinical outcome. Their condition may deteriorate quickly and soon develop cardiovascular collapse, multi organ failure and then death, if not resolved. According to some recently published studies, it has beenestablished that IL-6 is one of the key components inCOVID-19 related cytokine storm. IL-6 cytokine has a critical role in inflammatory as well as immune reactions. The therapeutic measures to optimize IL-6 levels are proven to be beneficial in critical patients. For this purpose, a monoclonal antibody against IL-6 receptor, Tocilizumab is recently recommended by National Health Commission of China, to counter the effects of this cytokine storm.[6]

The objective of the current study is to determine and evaluate the response of Tocilizumab with special reference to C- reactive protein (CRP) in critically ill patients with COVID-19.

## METHODS

This retrospective study included the data of 55 severe COVID-19 patients (respiratory rate ≥30, $SpO_2$ <93%, oxygen requirement ≥5L/min, $PaO_2/FiO_2 \leq 300$ mmHg) admitted in Corona unit of Farooq hospital West Wood Lahore, who were being treated with Tocilizumab along-with standard treatment protocol between April 27 and June 21, 2020. The data has been retrieved from hospital records of all patients who were treated with the standard intensive care (supplemental oxygen, dexamethasone, hydroxychloroquine, azithromycin, antivirals, ivermectin and low molecular weight heparin), along with Tocilizumab after taking permission from medical superintendent Farooq hospital West Wood, Lahore. It was given intravenously at 8 mg/kg bodyweight (up to a maximum of 800 mg) in two infusions, 12 hours apart. The primary endpoint was a composite of invasive mechanical ventilation or death. All the patients in this study were those who were treated with Tocilizumab. Demographic, clinical features and serum CRP were collected for each of these patients, before and after administration of Tocilizumab. The study was approved by the ethical committee of Farooq hospital West Wood, Lahore vide Letter No. FH/CU/02/2020 dated: 22.05.2020.

## STATISTICAL ANALYSIS

The data was analyzed using Statistical Package for the Social Sciences (SPSS) version 22.0. Mean and standard deviation were generated for continuous clinical and demographic variables and were represented in the form of a table (Table 1). Categorical variables were represented as frequencies and percentages. Shapiro-Wilk test was used to access normality in CRP levels – mg/L. As the P-values were less than 0.05 in most time intervals, non-parametric statistical tests were used. To see whether the intervention of Tocilizumab was successful or not in decreasing CRP levels, Friedman's 2-way ANOVA was used. Additionally, post hoc analysis with Wilcoxon signed-rank test was conducted with a Bonferroni correction applied, resulting in a significance level set at $P<0.017$. The results were summarized in the form of a line graph for better visual presentation

(see Graph 1). The CRP levels at different time intervals in COVID-19 patients who survived or died were compared using Independent Samples Mann-Whitney U Test. The results were represented both graphically and in tabular form.

## RESULTS

Out of 55 patients who were administered Tocilizumab, 72.7% (n=40) survived whereas 27.3% (n=15) died. Median (IQR) CRP levels (mg/L) at the time of admission, before Tocilizumab administration, and after Tocilizumab was administered, were 74.3 (33.6 to 98.0), 72.3 (26.1 to 94.2), 38.6 (22.2 to 57.2), respectively. There was a statistically significant difference in CRP levels before and after Tocilizumab was administered, $\chi^2(2) = 18.70$, $P<0.0001$. The pair-wise comparisons showed that there was no significant difference between CRP at the time of admission and Tocilizumab was administered ($Z=1.477$, $P=0.14$). However, there was a statistically significant difference between CRP level at the time of admission and CRP level 72 hours after Tocilizumab was administered ($Z=4.424$, $P<0.0001$). There was also a statistically significant difference between CRP before Tocilizumab was administered and CRP 72 hours after Tocilizumab was administered ($Z=4.147$, $P<0.0001$). The comparison of CRP levels in COVID-19 patients who survived or died showed that there were no statistically significant median differences of CRP levels at the time of admission, before and post-administration of Tocilizumab, respectively. Nevertheless, the descriptive statistics suggest (see **Table-2** and **Fig. 2**) that there was higher median reduction of CRP levels in patients

**Table-1:** Clinical and demographic characteristics of Coronavirus Disease 2019 (COVID-19) patients (mean ± SD)

| | |
|---|---|
| **Gender** | |
| Male | 48 (87.3%) |
| Female | 7 (12.7%) |
| **Age(range) in years** | 57.6 ± 13.6 |
| Male | 57.1 (30-83) |
| Female | 61.4 (50-75) |
| **Vitals** | |
| Temperature | 98.7 ± 1.2 |
| Blood pressure systolic | 122.4 ± 13.8 |
| Blood pressure diastolic | 76.2 ± 7.5 |
| Respiratory rate | 22.7 ± 3.2 |
| $SO_2$ | 92 ± 4.0 |
| ***RT-PCR at the time of admission** | |
| Negative** | 9 (16.4%) |
| Positive | 46 (83.6%) |

*Reverse transcriptase polymerase chain reaction PCR was negative**at the time of admission which became positive 2 days after admission on re-testing)

**Table-2:** Comparison of CRP levels – mg/L in Coronavirus disease 2019 (COVID-19) patients who survived or died.

| CRP | Survived (n = 40) | Died (n = 15) | p-value |
|---|---|---|---|
| At admission | 77.5 (34.3 – 102.8) | 65.5 (22.8 – 82.5) | 0.21 |
| Pre-Tocilizumab | 75.3 (26.8 – 100.3) | 70.6 (22.8 – 82.5) | 0.43 |
| Post-Tocilizumab | 34.9 (21.1 – 55.0) | 45.3 (22.8 – 58.1) | 0.35 |

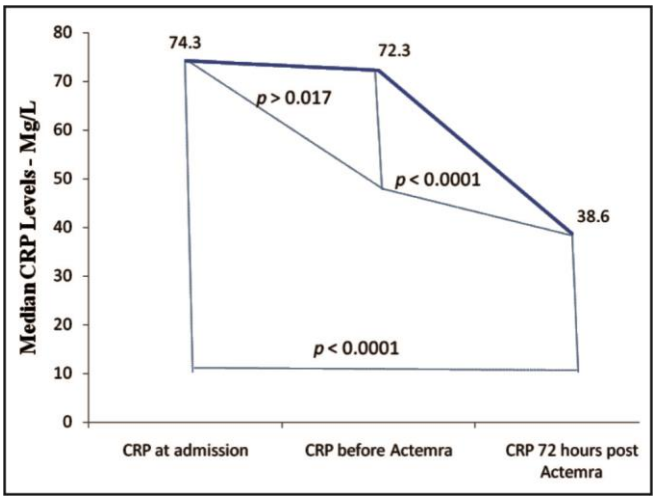


**Fig.1:** Median CRP levels at admission, pre and post administration of Tocilizumab (Actemra).

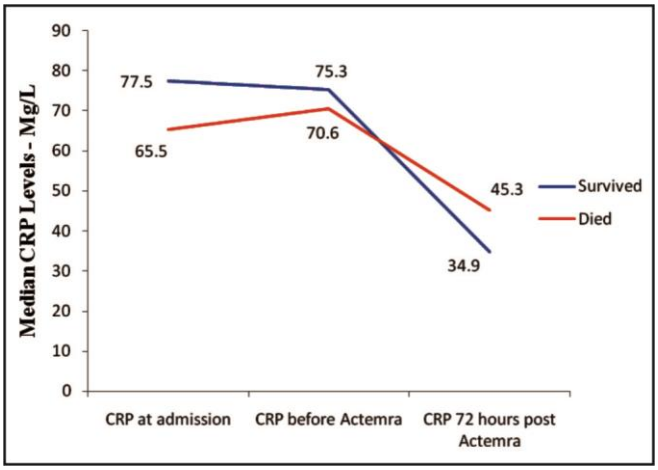


**Fig.2:** Median CRP pattern according to outcome.

who survived (77.5 to 34.9 mg/L) as compared to those who died (65.5 to 45.3 mg/L).

Results are shown as median and interquartile range (between brackets).

## DISCUSSION

In the present study, the effects of Tocilizumab therapy in COVID-19 pneumonia patients in response to CRP levels are discussed. Findings of the current study supported the use of Tocilizumab in the treatment of cytokine storms produced by COVID-19 as there was a statistically significant difference in CRP level before and after Tocilizumab a was administered (P<0.0001).

CRP is an acute-phase protein that is produced by the liver because of inflammation and tissue damage. The natural history of severe COVID-19 pneumonia is thought to be driven by a so-called cytokine storm[7]characterized by production of cytokines in increased numbers causing lung tissue damage and fibrosis.[6] Interleukin-6 (IL-6) is believed to initiate CRP release, although other acute systemic phase response cytokines like tumor necrosis factor alpha (TNFα) and interleukin-1 are also involved.[8]

The CRP secretion begins within 4-6 hours of the start of initiation of tissue inflammation, going to be doubled after every 8 hours and reaches its peak around 36-50 hours. After the elimination of the stimulus, the CRP level rapidly declines, having half-life of 19 hours. If, the causative stimulus remains there then the CRP level remain high, for extended period.[9] CRP measurement is regarded as highly valuable prognostic marker of inflammation and sepsis. It also aids in monitoring the response of therapy in patients. Studies show that after third day of successful treatment there is a decrease in the CRP level suggesting it as a good prognostic marker.[10,11] No therapy still has been approved for COVID-19 pneumonia, but recent clinical approaches deem the use of combination of antibiotics, antiviral drugs and immunomodulatory drugs including tocilizumab, a recombinant humanized monoclonal antibody against the interleukin-6 receptor.. Tocilizumab, have been tested in clinical care for the treatment of severe COVID-19 pneumonia.[12,13,14]

A single canter study by Pan Luo et al.[6] from Wuhan, China, showed a clinical benefit in patients with COVID-19 pneumonia with risk of cytokine storm. Similarly, we found a significant reduction in CRP level of patients after 72 hours of treatment with IL-6 antagonist, tocilizumab.

Although comparison of CRP levels in COVID-19 patients who survived or died showed no statistically significant median differences of CRP levels at the time of admission, before administration of Tocilizumab and post-administration of Tocilizumab, respectively in current study. But the significant reduction in CRP was observed in patients with severe COVID-19 pneumonia in current study after they were treated with tocilizumab and standard of care. As results suggest (Table-2 and Fig. 2) that there was higher median reduction of CRP levels in patients who survived (77.5 to 34.9 mg/L) as compared to those who died (65.5 to 45.3 mg/L). Our results are consistent with French case-control study done by Klopfenstein and colleagues,[13] in which mortality rate or need for mechanical ventilation were higher in patients who had not received tocilizumab than those who did (72% *vs.* 25%; P=0·002). The CORIMUNO randomized clinical trial, has also shown a favorable effect of tocilizumab when compared with standard of care.[15] Study done in Italy by G Guaraldi et al.[16] showed that 73 (20%) patients in the standard care group has died in comparison to 13 (7%) with a statistical value of (P<0·0001) patients treated with tocilizumab.

Tocilizumab, administered intravenously or subcutaneously, can be considered as one of the immunomodulatory drugs that has been tested in clinical care for the treatment of severe COVID-19 pneumonia.[12,13,14,17]

## CONCLUSION

Tocilizumab administration along with serial CRP monitoring might be helpful in reducing the complications of cytokine storm in patients with severe COVID-19 pneumonia. Although these results are encouraging, they should be confirmed in ongoing randomized studies.

## LIMITATIONS OF STUDY

The results of current study should be evaluated with caution as it was an open label study because blind trial was not possible. The strengths of this present study are that it is the first study that included patients from a real-life hospital setting from Pakistan on Tocilizumab therapy. Although we reported a good response in patients with Tocilizumab but studies from multiple centers with larger number of patients are required to make a definitive conclusion. Tocilizumab use in severe COVID-19 pneumonia is still in its infancy, as many questions are still open and the best treatment protocols have yet to be developed.

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## CONFLICT OF INTEREST

None to declare.

## FINANCIAL DISCLOSURE

None to disclose.

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### *Author's Contribution*

**AM:** Acquisition and analysis of data and drafting of article.

**OF:** Conception and design of study.

**SW**: Study design, interpretation of data and drafting of manuscript.

**MK:** Design and acquisition of patient data.

**AM:** Drafting the manuscript and revision with important intellectual content.

**MA:** Critical revision of the manuscript for intellectual content.

**MU:** Acquisition of data.

**ALL AUTHORS:** Final approval of the version to be published.