RESEARCH ARTICLE

The Effect of Tocilizumab on the outcome of patients with severe COVID-19 with cytokine storm syndrome in a sample of Iraqi patients

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Abstract

Objective: To determine whether tocilizumab improved outcomes of patients hospitalised with severe coronavirus disease-2019 cytokine storm syndrome.

Method: The case-control study was conducted at Al-Yarmouk Teaching Hospital, Baghdad, and Al Shatra General Hospital, Thi Qar, Iraq, from September 2020 to March 2021, and comprised patients with severe acquired respiratory syndrome-corona virus-2 pneumonia who were not candidates for mechanical ventilation and received a single-dose intravenous infusion of tocilizumab 8mg/kg in group A. The outcomes were compared with patients in group B who received only standard care. Data was analysed using SPSS 26.

Results: Of the 60 patients, 30(50%) were in group A; 22(73.3%) males and 8(26.7%) females with mean age 56.63±10.92 years. There were 30(50%) patients in control group B; 24(80%) males and 6(20%) females with mean age 54.8±6.18 ($p>0.05$). Group A showed significant changes compared to group B in the levels of interleukin-6, serum ferritin, D-dimer, procalcitonin, lymphocytes count and oxygen saturation ($p<0.05$). Mortality rate was not significantly different between the groups ($p>0.05$).

Conclusions: Majority of the acute phase inflammatory markers were reduced significantly by treatment with tocilizumab.

Key Words: Tocilizumab, fibrin, Interleukin, Procalcitonin, Cytokine, Infusions, Intravenous, Oxygen Saturation, Respiration, Artificial, Coronavirus, Lymphocytes, Ferritins

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Introduction

Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), or 2019 novel coronavirus (2019-nCoV), coronavirus disease-2019 (COVID-19) is an enveloped non-segmented positive-sense ribonucleic acid (RNA) virus from the Coronaviridae-order Nidovirales family[1,2].

The clinical picture is characterised by heterogeneous symptoms ranging from mild fatigue to life-threatening pneumonia, systemic inflammatory reaction, acute respiratory distress syndrome (ARDS) and multi-organ failure (MOF)[3].

Viral particles shedding from the respiratory mucosa induce inflammatory reaction from other cells in the body through a series of immunological reactions that induce cytokine release, culminating in a cytokine storm. The propensity of the immune reaction determines the severity of the clinical syndromes[4].

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Therefore, prompt diagnosis and management of critically ill patients with SARS-CoV-2 pneumonia are vital because the mortality is high, especially in older patients with comorbidities[4,5]. SARS-CoV-2 pneumonia places greater pressure on hospital critical care resources, especially in hospitals that are not adequately staffed or have limited resources[5].

The exact mechanisms of severe lung injury and MOF are yet to be elucidated. There is an interplay of non-resolving immune hyper-inflammatory systemic response and inflammatory marker surplus because of the ongoing viral replication (cytokine storm) with ARDS[6-8].

Differences in host immune-related responses in different patients with variable symptomatic severities suggest the possible role of host immune dysregulation[7].

The current interaction created the base for the highly targetting immunomodulatory therapies using particular cytokine antagonists to block pathways critical to host immune responses[6, 7].

Cytokines play a key role in coordinating antimicrobia1 effector ce1ls and providing regulatory signals that direct, amplify and resolve the immune response. Cytokine storm is a term encompassing several disorders of

immune dysregulation characterised by constitutional symptoms, systemic inflammation and multi-organ dysfunction that can lead to MOF if inadequately treated. Interferon-gamma (γ), interleukin-1 (IL-1), IL-6, tumour necrosis factor (TNF) and IL-18 contribute to the immunopathogenic process of cytokine storm[7].

IL-6 is a marker of T-cell activation[7], and a major inducible pro-inflammatory cytokine which affects different organ systems through immunological and non-immunological pathways[7,9]. IL-6 is one of the most robust prognostic markers in lung injury, end-organ damage, and subsequent survival[7,10].

Elevation of the immune markers that reflect hyper-inflammation and tissue damage predicted worsening outcomes in patients with severe infection with SARS-CoV-2[11]. However, it is still unknown whether the cytokine storm is a primary event of the infection, or an unpredicted secondary process[7].

The SARS-CoV-2-associated cytokine storm differs from other cytokine release syndromes due to other diseases.

From the diagnostic point of view, patients with SARS-CoV-2-associated cytokine storm have more lymphopenia[7], frequent thromboembolic events[12] and higher IL-6 and other inflammatory biomarkers[8].

Theoretically, implementing agents to block the cytokine signalling may deteriorate SARS-CoV-2 clearance, increase the risk of secondary infections, and consequently worsen the outcomes[13]. However, IL-6 blockade also results in a rapid decline of serum IL-10, an immunosuppressive cytokine secreted by macrophages, which may alleviate concerns about prolonging viral clearance[14].

Tocilizumab (TCZ) is a recombinant humanised immunoglobulin antihuman IL-6 monoclonal antibody approved for the treatment of many immune-mediated rheumatological conditions[15], Castleman disease[16], Crohn's disease[17] and steroid-refractory chronic graft-versus-host disease[18].

TCZ prevents binding of IL-6 to both cell-associated and soluble IL-6 receptors with high affinity inhibiting the IL-6 signalling[15], rendering it incapable of immune damage to target cells and alleviating the inflammatory responses [19], which is the mechanism that made TCZ of benefit in severe or life-threatening chimeric antigen receptor T cell-induced cytokine storm[20].

Blocking the IL-6 may halt the disturbed oxygen diffusion through the alveolar-capillary blood-gas exchange dysfunction. An additional benefit could be seen through the lesser frequency of pulmonary fibrosis and organ failure, which may be seen in critically ill patients[21]. Still, there was no survival advantage using TCZ in severely ill hospitalised patients with SARS-CoV-2 in many studies[22-24].

The current study was planned to determine whether TCZ improved outcomes of patients hospitalised with severe COVID-19 cytokine storm syndrome.

Patients and Methods

The case-control study was conducted at Al-Yarmouk Teaching Hospital, Baghdad, and Al Shatra General Hospital, Thi Qar, Iraq, from September 2020 to March 2021, and comprised hospitalised patients with SARS-CoV-2 pneumonia who were not candidates for mechanical ventilation. After approval by ethics review committee of the Iraqi Board of Medical Specialisation, the sample size was calculated using G*Power software [25] with 95% confidence interval (CI) and 5% margin of error.

Those include were patients of either gender aged >18 years who had positive polymerase chain reaction (PCR) test and positive radiological findings on computed tomography (CT) imaging of the chest.

Those with a previously documented hepatic and renal dysfunction, with a previously documented thromboembolic diseases of any severity, patients with any type of malignancies regardless of their treatment status, patients with documented active or chronic inflammatory conditions, patients who received any immune modulation therapies in the preceding 6 months, patients on regular steroid doses in the preceding 6 months, and pregnant women were excluded.

After taking written informed consent, the patients enrolled were divided into cases group A and control group B. The sample was followed throughout the respective admission period and their progress was assessed.

As per the institutional practice, the patients in both groups received standard of care (SOC), which included antiviral treatment, if available, systemic glucocorticoids, and supportive care.

In addition to SOC, group A patients received Intravenous (IV) TCZ 8mg/kg body weight, to a maximum of 800mg/dose, once. Efficacy was evaluated before TCZ administration and at discharge. Patients who were candidates for discharge from hospitals had to be fever-free for >3 days, with marked improvement in their

respiratory functions, and SARS-CoV-2 PCR test negative twice with a sampling interval of one day.

Laboratory assessment included inflammatory markers IL-6, C-reactive protein (CRP), lactate dehydrogenase (LDH), D-Dimer, procalcitonin, serum ferritin, complete blood picture (CBC) parameters haemoglobin (Hb), white blood cells (WBC), neutrophil, lymphocyte and platelet counts. Neutrophil-to-lymphocyte ratio (NLR) was calculated by dividing the neutrophil count by the lymphocyte count. For patients who died, mortality was taken as the outcome.

Venous blood samples 6-8mL in 2 test tubes were drawn at baseline and at discharge from all the participants. Part of the sample (2-3 mL) was taken in ethylenediaminetetraacetic acid (EDTA) tube for CBC, gently inverted 8-10 times at room temperature which was tested later (Cell-Dyn-Ruby, Germany) within an hour.

The rest of the sample (4-5mL) was taken in a plain tube for biochemical investigations (Beckman-Coulter-Unicell-DXC-600-Synchron Clinical System, Beckman Coulter Inc., Brea, California, US, or Biolyzer-300, Analyticon Biotechnologies AG, Lichtenfels, Germany).

Data was analysed using SPSS 26. Data was expressed mean ± standard deviation (SD), median ± standard error (SE), or frequencies and percentages, as appropriate. Bivariate correlation analysis was used to compare the relationships between the variables at admission and discharge using Spearman's correlation coefficient, with values >0.3 being the minimally accepted values. Independent sample t-test was used to study the effect of the change in the biochemical and the clinical parameters in the study groups. The relationship between mortality as a dependent outcome variable and the biochemical and clinical changes during hospitalisation was assessed using non-parametric Mann-Whitney-Wilcoxon test and the independent sample t-test. Two-sided asymptotic significance level of ≤0.05 was used. Spearman's correlation coefficient (rho) significance levels can be measured even if the data did not achieve normal distribution. In subgroup analysis, the exact significance was used which was equal to 2*(1-tailed significance).

Results

Of the 60 patients, 30(50%) were in group A; 22(73.3%) males and 8(26.7%) females with mean age 56.63±10.92 years. There were 30(50%) patients in control group B; 24(80%) males and 6(20%) females with mean age 54.8±6.18 (p>0.05) (Table 1).

All the measured variables showed significantly moderate to strong effect size, except CRP, which failed to show any significance, while LDH showed the highest significance correlation (Table 2).

Table-1: Baseline characteristics. n (%) or mean±SE.

Parameters	Cases (n=30)	Controls (n=30)
Gender (males) n (%)	22 (73.3)	24 (80)
Age (years)	56.63±10.92	54.8±6.18
Body mass index (kg/m^2)	24.58±3.39	27.08±4.13
Duration of hospitalization in days	10.77±4.87	10.43±3.76
Hypertension n (%)	5 (16.7)	11 (36.7)
Ischaemic Heart Disease n (%)	3 (10)	4 (13.3)
Interleukin-6 (pg/mL)	85.12±7.31	38±4.28
CRP (µg/mL)	148.5±8.48	140.15±9.1
LDH (U/L)	607±59.73	655.5±60.18
D-Dimer (ng/mL)	666±208.79	1176±412.85
Serum Ferritin (ng/mL)	721±70.12	649.1±38.83
Procalcitonin (ng/mL)	0.1±0.033	0.2±0.14
Haemoglobin (g/dL)	14.3±0.26	13.25±0.25
WBC (µL*10^3)	24.61±16.52	10.52±0.65
Neutrophils (µL*10^3)	5.9±0.71	10±0.68
Lymphocytes (µL*10^3)	0.7±0.1	0.5±0.08
Platelets (µL*10^3)	259±13.28	190.5±15.22
NLR	10.06±1.54	27.04±4.69
SPO2 without 02 %	0.90±0.01	0.84±0.01
SPO2 with 02 %	0.91±0.01	0.89±0.01
Received antiviral medication n (%)	13 (43.3)	10 (33.3)
Mortality n (%)	4 (13.3)	5 (16.7)

CRP: C-reactive protein, LDH: Lactate dehydrogenase, WBC: White blood cells, NLR: Neutrophil-to-lymphocyte ratio, SPO2: Partial pressure of oxygen.
Data is expressed as n(%) or mean ± standard error (SE).

Markedly significant changes were noted for IL-6, serum ferritin, D-dimer, procalcitonin, lymphocyte count and partial pressure of oxygen (SPO2), while changes in the other variables were non-significant (Table 3).

Mortality was the outcome in 4(13.3) group A patients and 5(16.7) group B patients. There was no significant difference with any of the variables in intergroup or intragroup analysis (Table 4).

Discussion

The current case-control study was age-matched with noticeable male preponderance. Earlier reports during the epidemic confirmed the relationship between older age and poor outcomes in SARS-CoV-2 infection, but did not identify separate effects of gender and obesity on prognosis[2,5,11,22,26], yet, some studies showed certain male preponderance[4,11,27,28]. Different results may be related to the insufficient number of samples and mode of spread of the disease.

In most patients, acute-phase reactant levels decreased and the patients were getting to a stable condition as reflected by a later gradual decreased of IL-6 or other

Table-2: Bivariate Correlation of biomarkers at admission and at discharge.

Variables	Cases	Control	Spearman's (rho) Correlation Coefficient	Sig.(2-tailed)
Interleukin-6				
Admission	96.83	43.25	0.50	<0.001
Discharge	37.73	30.47		
CRP				
Admission	137.25	139.11	-0.16	0.224
Discharge	78.34	103.96		
Ferritin				
Admission	809.87	674.84	0.63	<0.001
Discharge	579.54	640.14		
LDH				
Admission	726.47	696.37	0.79	<0.001
Discharge	543.4	543.00		
D-Dimer				
Admission	1189.98	2035.59	0.57	<0.001
Discharge	1000.25	1259.84		
Procalcitonin				
Admission	0.16	0.53	0.48	<0.001
Discharge	0.18	0.23		
WBC				
Admission	24.61	10.52	0.58	<0.001
Discharge	10.18	12.58		
Lymphocyte				
Admission	0.88	0.58	0.48	<0.001
Discharge	1.24	0.58		
Neutrophils				
Admission	6.81	9.55	0.61	<0.001
Discharge	8.41	11.39		
Platelets				
Admission	236.7	207.87	0.47	<0.001
Discharge	274.63	231.2		
Haemoglobin				
Admission	14.16	13.44	0.68	<0.001
Discharge	13.83	12.85		
NLR				
Admission	10.06	27.04	0.66	<0.001
Discharge	12.92	28.95		
SPO_2 without O_2				
Admission	0.88	0.83	0.45	<0.001
Discharge	0.91	0.89		
SPO_2 with O_2				
Admission	0.91	0.89	0.45	<0.001

CRP: C-reactive protein, LDH: Lactate dehydrogenase, NLR: Neutrophils-to-lymphocyte ratio, SPO2: Partial pressure of oxygen, WBC: White blood cells.

acute inflammatory markers after TCZ administration compared to the control group, which was similar to earlier results[29-31].

IL-6 showed significant difference in the treatment group in comparison to the SOC group, but this difference did not show significant correlation with mortality.

Therapeutics targeting the patient immune response to viral infection was proposed as a potential strategy to ameliorate inflammation and improve outcomes in severe SARS-CoV-2 illness[31].

A study showed that TCZ was significantly beneficial in patients with IL-6 concentration around 10-90 times above the normal range[29].

The pre-treatment levels of IL-6 were expected to increase in severe cases of SARS-CoV-2, which could induce lung epithelial cells to increase inflammatory responses, leading to increased macrophage response and ultimately pulmonary damage[30].

Table-3: Non-parametric testing for the assessment of changes in the study parameters between the study groups.

Variables	Mann-Whitney U	Wilcoxon W	Z	Asymp. Sig. (2-tailed)
Δ Interleukin-6	85	550	-5.397-	0.001
Δ CRP	369	834	-1.198-	0.231
Δ Ferritin	242	707	-3.075-	0.002
Δ LDH	401	866	-.717-	0.473
Δ D-Dimer	310	775	-2.070-	0.038
Δ Procalcitonin	174.5	639	-4.243-	0.001
Δ WBC	444.5	909	-.081-	0.935
Δ Lymphocytes	273	738	-2.630-	0.009
Δ Neutrophils	449	914	-.015-	0.988
Δ NLR	439	904	-.163-	0.871
Δ Platelets	435.5	900.5	-.214-	0.830
Δ SPO_2 without O_2	222	687	-3.394-	0.001
Δ SPO_2 with O_2	188	653	-3.892-	0.001

CRP: C-reactive protein, LDH: Lactate dehydrogenase; NLR: Neutrophils-to-lymphocyte ratio, WBC: White blood cells, SPO2: Partial pressure of oxygen.

Table-4: Nonparametric testing related to mortality between the groups.

Variables	Cases vs. Controls Asymp. Sig. (2-tailed)	Cases only Exact Sig. [2*(1-tailed Sig.)]
Δ Interleukin-6	0.967	0.930
Δ CRP	0.605	0.461
Δ Ferritin	0.598	0.837
Δ LDH	0.182	0.659
Δ D-Dimer	0.541	0.461
Δ Procalcitonin	0.444	0.391
Δ WBC	0.291	0.391
Δ Lymphocytes	0.275	0.791
Δ Neutrophils	0.268	0.328
Δ NLR	0.061	1.000
Δ Platelets	0.286	0.123
Δ SPO_2 without O_2	0.518	0.157
Δ SPO_2 with O_2	0.328	0.702

CRP: C-reactive protein, LDH: Lactate dehydrogenase; NLR: Neutrophils-to-lymphocyte ratio, WBC: White blood cells, SPO2: Partial pressure of oxygen

There would be temporary increase in the initial post-treatment levels due to the IL-6R blockage by TCZ, followed by gradual reduction in the levels. Stabilisation and improvement in clinical outcomes were demonstrated following the inhibition of inflammatory activity by TCZ[21, 24, 29].

The IL-6 levels may remain unchanged after TCZ administration, which can be because TCZ competitively blocks IL-6 receptors and leaves free IL-6 in plasma[32], and inhibits the receptor-mediated clearance of IL-6, leading to its accumulation in serum[29].

Many studies considered IL-6 as independent, significant, robust prognostic predictor of disease severity and death, which should be considered in the management of patients with SARS-CoV-2 infection to stratify prospective clinical trials, guided resource allocation and informed therapeutic options. It is possible that patients with moderate disease and high IL-6 levels will benefit the most from cytokine blockade by TCZ[10, 33, 34]. Higher concentrations of IL-6 in serum are associated with higher levels of SARS-CoV-2 viremia[33].

CRP levels reduced during the course of the current study, but the association between admission and discharge CRP values did not show any significant association during overall analysis or even in subgroup analysis. Its change in mortality analysis was not significant, which was in contrast to other studies, possibly due to the markedly low sample size[35 - 37].

Low lymphocyte counts, high concentration of CRP, LDH and D-dimer were observed in the majority of SARS-CoV-2 patients. These biomarkers were found to be significantly correlated with disease severity and subsequent mortality[35, 36].

Researchers have proposed using CRP or other inflammatory markers to select patients with hyper-inflammatory state for TCZ treatment[22, 37]. Respiratory dysfunction is a more useful predictor of which patients might benefit from IL-6 inhibition[38].

D-dimer levels and platelet count were significantly different between the TCZ and SOC groups in the current study. Still, their effect on mortality could not be ascertained in either group.

Thachil et al. showed that measuring D-dimers and platelet count in all SARS-CoV-2 patients may help in identifying patients who may need admission and close monitoring [39]. Tang et al. reported that non-survivors had significantly higher D-dimer levels compared to the survivors[40]. Thrombocytopenia is often considered an indicator of sepsis mortality[34]. High levels of D-dimers have been reported in several cohorts of patients with SARS-CoV-2, and correlated with increased mortality[41].

Levels of LDH and procalcitonin were markedly high at the pre-treatment stage, and underwent significant reduction subsequently. The difference, however, did not reach the significance level and did not affect mortality at any level.

Many studies noted elevated LDH during the initial phases of the illness[26, 27, 37, 42], and such levels were taken as markers for mortality of patients with SARS-CoV-2 infection[42, 43].

The increase of the serum levels of cell death markers, such as LDH and D-dimers, may be diagnostically important in impending cytokine storm, which is characterised by significant systemic tissue damage[11]. The elevated levels of these markers, especially early on, could also indicate pulmonary immunothrombosis and secondary pulmonary arterial hypertension due to offending infection [32].

Studies showed conflicting results regarding prognostic value of procalcitonin level in severe cases of SARS-CoV-2[27, 44].

The cellular inflammatory responses were augmented at the initial stages during the study, and the difference during therapy with TCZ took different correlations to the outcome.

There is a complex mechanism for the neutrophils action in patients with SARS-CoV-2. Viral invasion produces different degree of changes in circulating cell counts, and that is most likely due to a complex interplay among cytokine-induced changes in production and mobilisation of cells from the bone marrow, immune-mediated destruction, and chemokine-induced migration. It is customary to find some patients with neutrophilia, and others with neutropenia, although they had the same degree of severity of the illness[7, 26].

Wang et al. showed that among the non-survivors, the neutrophil count, blood urea, creatinine levels and D-dimer continued to increase, and the level of lymphocyte continued to decrease until death occurred[26].

A study showed lower rates of serious infections in patients who received TCZ, even with the decreased neutrophil count and increased rates of neutropenia [23, 27]. The mechanism of lymphopenia and neutropenia may be due to the direct cellular effect by the viral invasion, or indirect as a response to the excessive cytokine release [26,27].

After TCZ therapy, temporary neutropenia may be encountered and resolved after discontinuation of the TCZ. The mechanism of neutropenia is the margination effect rather than myelo-suppression[15, 20].

Severe viraemia may trigger a series of complex immune dysregulatory responses, and results in changes in immune components such as peripheral blood leukocytes and lymphocytes, inhibiting their function[45]. The viral particles damage the cytoplasmic components of the lymphocytes, and cause its destruction and accelerate apoptosis, which may contribute to the progression of the disease[5, 46].

Previous studies showed lymphocytopenia in most patients of different grades, with unclear relation between the severity of lymphocytopenia and the severity of SARS-CoV-2 infection.[4,5,26,36,42,43,46] Some studies suggested that lower lymphocyte count could be associated with higher mortality in such patients[42,43].

Additionally, high NLR has been recognised as a useful prognostic haematological marker for the early screening of SARS-CoV-2 severity[36,47]. NLR was introduced as a useful parameter for monitoring disease activity in systemic inflammatory diseases[48-50].

The ΔNLR was considered as a surrogate marker for predicting bacterial infections in patients with rheumatoid arthritis (RA) treated with TCZ[51], and a parameter to monitor the clinical response to TCZ[52].

Studies showed that blockade of IL-6 receptors by TCZ administration produced an important effect on the systematic inflammatory response expressed as decreased values of CRP and NLR[51,53].

Oxygen therapy during the study was governed by the development of hypoxia. The current study showed significant improvement in SPO2 in patients in both groups. This difference did not reach the level at which an effect on mortality could be seen. The overall mortality was 15%.

High rate of in-hospital mortality was shown for severe cases of SARS-CoV-2. There was a logical significant association between mortality and hypoxia in many studies, ranging 35-62% in critically ill patients.[5,31,54, 55]

Individualisation (not selectivity) of care was highly applied for patients during the epidemic in terms of the need for invasive ventilatory support in severe cases, which may be specific for the first two weeks of the illness[5,26], length of the hospitalisation and the criteria for discharge[27].

Despite the fact that the current study was conducted at two centres, it had several limitations. The main limitation was the TCZ availability, which limited the sample size. False negative cases by PCR for SARS-CoV-2 were excluded from the study. Many patients refused receiving TCZ due to misinformation or fake news. Information about possible timing and mode of transmission could not be ensured, that is why the study could not gather information about the incubation period. The causal association between the clinical outcome and the changes in different clinical and laboratory parameters could not be ascertained owing to the observational design of the study. Also, unmeasured residual confounding could not be ruled out. The study was also open label, so the staff involved knew which patients were receiving TCZ. The generalizability of the results must be considered in relation to different epidemiological settings, especially regarding the TCZ dose and use at the appropriate time point of the disease course. Finally, the study lacked longitudinal data for follow-up post-discharge. The fact that the majority of acute-phase inflammatory markers were reduced significantly during the course of treatment with TCZ compared to SOC, but there was no difference in mortality between the groups might have been because of the small sample size and the short follow-up. Randomised controlled clinical trials with larger sample sizes are recommended to further investigate the effects of TCZ in patients with severe COVID-19 and cytokine storm syndrome.

Conclusion

Majority of the acute-phase inflammatory markers reduced significantly during the course of treatment with TCZ compared to the SOC group.

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