Efficacy of Tocilizumab on Critical Intensive Care Unit COVID-19 Patients: Retrospective Study Among Egyptian Armed Forces Hospitals

Original Article

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ABSTRACT

Background: Since the first reports of COVID-19 disease, it has become clear that acute respiratory distress syndrome was responsible for a significant number of deaths among infected patients. Several studies have linked increased levels of proinflammatory cytokines in serum to pulmonary inflammation and extensive lung damage. Among proinflammatory cytokines, Interleukin-6 is the key cytokine that contributes to an inflammatory storm. Several studies have found that elevated IL-6 levels are associated with the severity of COVID-19 symptoms and death. Tocilizumab is monoclonal antibody that antagonizes the effect of IL-6. Early cytokine storm treatment may have benefits on COVID-19 patients' survival.

Patients and Methods: The present study was conducted on 91 COVID-19 patients, admitted to intensive care unit and took tocilizumab. The IL-6 serum level data were collected from patients' files pre and post-tocilizumab administration. Regarding these data, we could determine the clinical outcome after treatment.

Results: We demonstrated that there was a decrease in IL-6 serum level within both mild and moderate severity grades by 16.3% and 38.4% respectively, while there was a significant increase in the patients who had severe grade by 162%. There was a significant decrease in IL-6 serum level within recovered group by 66.1% and a significant increase within mortality group by 256% pre and post-injection with tocilizumab.

Conclusion: IL-6 serum level may be a major indicator but not the sole driver in the pathology of COVID-19. So, when we assess IL-6 serum level, we should take into consideration other cytokines, and usage of combinations of many blockages for these cytokines may improve the outcome.

Key Words: COVID-19, Cytokine storm, IL-6, IL-6 antagonist, Tocilizumab.

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INTRODUCTION

Previous coronavirus outbreaks have included Middle East Respiratory Syndrome (MERS) and Severe Acute Respiratory Syndrome (SARS) & both of them have been identified as agents posing a significant threatening on general health. In late December 2019 in Wuhan, China, a group of people was admitted to hospitals with a primary diagnosis of pneumonia of unknown aetiology. Given the estimated multiplication of the number for the 2019 Novel (New) Coronavirus, early studies projected the commencement of a potential Coronavirus outbreak[1]. Several studies have linked increased levels of proinflammatory cytokines in serum to pulmonary inflammation and extensive lung damage[2].

Coronaviruses (CoVs) are enveloped, positive sense, ssRNA viruses with a genome size (26k to 32k) nucleotides. They infect humans and animals & have been linked to a variety of respiratory, renal, GIT, and neurological diseases. They are classified into four families: alpha, beta, gamma, and delta. The Alpha, Beta, and Delta genera cause human infection, and infect upper respiratory tract but also infect lower respiratory tract in some cases[3].

The term "cytokine storm wide spreading among the normal people which refers to a condition in which the immune system is extremely activated. Cytokine storms characterise a extensive range of infectious and non-infectious diseases[4]. Among proinflammatory cytokines, GM-CSF and Interleukin 6 (IL-6) are the key cytokines that contribute to an inflammatory storm[5].

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Interleukin 6 (IL-6) is a cytokine with a numerous functions, including inflammation, autoimmunity effect, and acute phase response. It influences host defence via variety of immune-stimulating mechanisms[6].

IL-6 binds to the IL-6 receptor (IL-6R) forming a complex and this complex binds to transmembrane protein called glycoprotein 130 (gp130), resulting in the initiation of intracellular signal transduction[7]. Several studies have discovered that elevated interleukin-6 levels are associated with severe COVID-19 and are related to an increased likelihood of needing mechanical ventilation and death[8].

When IL-6 binds to hepatocytes, it causes a wide range of acute phase proteins to be produced, including C-Reactive Protein, complement C3, fibrinogen, thrombopoietin, and 1-antichymotrypsin, considering that IL-6 a key for acute phase response mediator. Serum iron levels are reduced as a result of IL-6-induced hepcidin synthesis, which inhibits the function of the iron transporter ferroportin 1 on the gut. IL-6 stimulates hematopoietic stem cell differentiation as well as megakaryocyte maturation, both of them culminate in platelet release. IL-6 impacts the direction of specific differentiation of naïve CD4 T cells in the acquired immune response while also promoting the development of activated B cells into Ig-producing cells. TGF-b is essential for T helper (Th) 17 development in combination with IL-6, while IL-6 reduces TGF-b-induced regulatory T-cell (Treg) differentiation[9].

Increased IL-6 serum levels are related with a poor prognosis and worsening clinical fate in COVID-19 patients. It has been demonstrated that somewhat higher IL6 levels more than 80 pg/ml are sufficient for identifying the increasing risk of COVID-19 patient for respiratory failure[10]. Additionally, serum RNAaemia of SARS CoV-2 closely related to cytokine storm and exceptionally increasing serum IL-6 levels[11].

Tocilizumab is a humanised recombinant monoclonal antibody of the IgG1 class that is act on the soluble and membrane-bound forms of the IL-6Rs[12].

Tocilizumab is now licenced to be used in the treatment of Castleman disease, rheumatoid arthritis (RA), and juvenile idiopathic arthritis (JIA). Furthermore, a number of off-label case studies and pilot trials of tocilizumab have given good results, signalling that tocilizumab may become established as a new medicine used in treatment of a variety of chronic intractable immune-mediated disorders[9].

Tocilizumab binding to cell-related IL-6R (macrophages, neutrophils, T-cells, etc.) and sIL-6R can prevent classical and trans-signals, thereby nhibits signal transduction through sIL-6R and mIL-6R. Tocilizumab is a humanised recombinant anti-human IL-6R IgG1 monoclonal antibody[13].

Proactive detection of serum acute phase reactants like IL-6 in COVID-19 patients may be used to monitor cytokine storm, which is a major cause of death. Early cytokine storm treatment may have maximum therapeutic advantages and a beneficial impact on COVID-19 patients' survival[7].

PATIENTS AND METHODS:

Study sample:

The present study is a randomized retrospective study which conducted at Almaza Fever Hospital ICU and treated by tocilizumab and Steroids. The study included confirmed Positive COVID-19 patients by PCR at Armed Forces Laboratories for Medical research and blood bank regardless their gender, age or comorbidities who administrated at Almaza Fever hospital ICU and treated by tocilizumab and Steroids between Sep 2020 and Apr 2021. We exclude patients who had any known immunological disorders, Patients on immunosuppressive drugs including tocilizumab to treat another disease and patients infected with HCV, HBV and T.B.

This study was done on 129 patients who were diagnosed as COVID-19 patients and admitted to intensive care units in Almaza fever hospital in the period from September 2020 to April 2021. The whole study group that the study was conducted on 129 COVID 19 diagnosed patients who were allocated to two groups, (group 1) who received tocilizumab with the treatment protocol for COVID-19, while (group 2) who didn't receive it.

Data collection:

The data collected from patient's files of Almaza Fever Hospital ICU and these data including: Full History taking including personal history and Co-morbidities (Such as diabetes Mellitus, respiratory disease, hypertension, cardiac diseases, Chronic renal disease and Chronic hepatic disease), General examination, Lab investigations, Imaging: Chest CT, Time to discharge from ICU and Outcome.

Procedures:

Definitive identification for SARS Cov-2 infection was confirmed by RT-qPCR according to the nationally suggested protocol, RNA extraction was performed on (Chemagic d 360-UK) by (Perkin Elmer - UK), the amplification and detection was performed on (QUANTI STUDIO 5 real time PCR-UK) by (Version 2 Thermofischer - UK) and it was accomplished according to the manufacturer's instructions.

IL-6 assay:-

IL6 test is a non-competitive (sandwich) chemiluminescent immunoassay done by Roche Elecsys; cells were harvested and blocked according to eBioscience protocols. The test claims an assessing range of 1.5 - 5000 pg/mL and the reference result less than seven (pg/mL).

Aim of the study:

The study aims to detect the level of serum IL-6 that will predict the efficiency of tocilizumab (survival) on critical ICU COVID-19 patients and to study the effect of tocilizumab on outcome and it is usage on reducing severe respiratory symptoms in critical ICU COVID-19 patients.

Data analysis:

The acquired data had been revised, coded, tabulated, and entered into the Statistical Package for Social Science (SPSS 26) on a PC. Data was given, and appropriate analysis was performed based on what category of data gathered for separate parameter. Descriptive statistics for parametric data include mean, standard deviation (SD), and range, whereas non-parametric data include median and interquartile range (IQR). Categorical data was expressed by rate of recurrence and proportion.

Analytical statistics:

Student-t, Mann Whitney (U test), Chi-Square, Fisher's exact, Repeated measure and One Way ANOVA & Post Hoc Test, Wilcoxon signed rank, The Receiver Operating Characteristic Curve (ROC) and Spearman's rho correlation analysis used to assess the relations between different variables considering p-Value significant at *<0.05*.

RESULTS:

This study was performed on 129 patients who were diagnosed with SARS-CoV2 and admitted to intensive care units in Almaza fever hospital in the period from September 2020 to April 2021.

Table (1): Shows demographic data for the whole study group that the study was done on 129 patients with SARS-CoV2 who were divided to two groups, (group 1) who received tocilizumab with the treatment protocol for COVID-19, while (group 2) who didn't receive it.

Table 1: Sociodemographic data of the within the studied groups:

		Mean ± SD N (%)
Age (Years)		51.39 ± 18.70
Group	Group 1	91 (70.5%)
Group 2	38 (29.5%)	
Sex	Male	92 (71.32%)
Female	37 (28.68%)	
Co-Morbidity		60 (46.5%)
Hospital Stay (Days)		13.52 ± 5.46
Outcome	Recovery	108 (83.7%)
	Mortality	21 (16.3%)

Table 2: Shows the CT severity score within the whole study group, which was divided to three groups.

Table 2: Chest CT severity score within the studied groups.

		N (%)
Chest CT severity score	Mild	40 (31.0%)
	Moderate	61 (47.3%)
	Severe	28 (21.7%)

Table (3): Shows demographic data between two study group, with significant difference in sex distribution and highly significant difference in age. There were no significant difference in patients who had co-morbidities nor in hospital stay.

Table 3: Relation between the two studied groups according the demographic data.

		Group		Test of significance	
		Group 1	Group 2		
		N (%) Mean ± SD	N (%) Mean ± SD	*p-Value*	Sig.
Sex	Male	59 (64.84%)	33 (86.84%)	*0.012**	S
	Female	32 (35.16%)	5 (13.16%)		
Age (Years)		56.21 ± 16.75	39.84 ± 18.25	*<0.001***	S
Co-Morbidity		46 (50.55%)	14 (36.84%)	*0.155**	NS
Hospital Stay (Days)		13.97 ± 5.54	12.45 ± 5.2	*0.151***	NS

*Chi-square test.
** Student t-test.

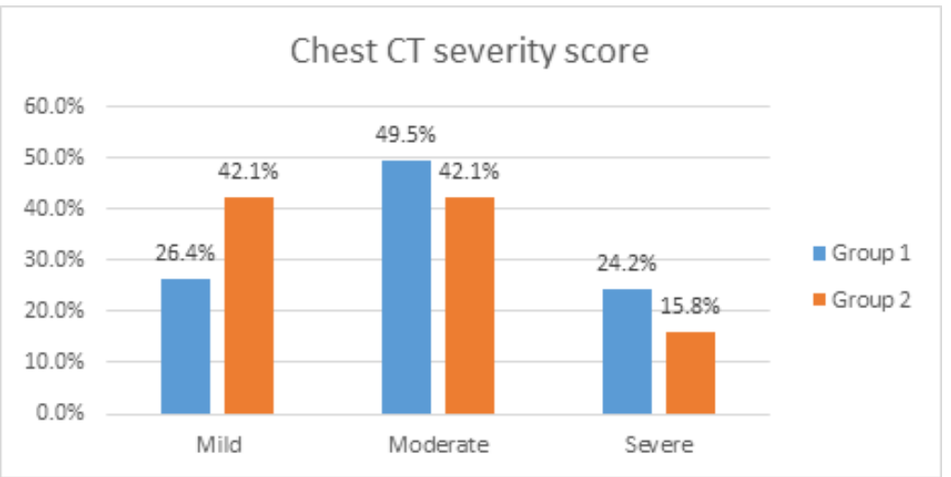

Fig. 1: Shows different grades of chest CT severity score between 2 studied groups, mild cases were more in group 2 than group 1, while moderate and severe cases were more in group 1 than group 2, without no statistical significant difference among the two groups as *p-Value > 0.05*.

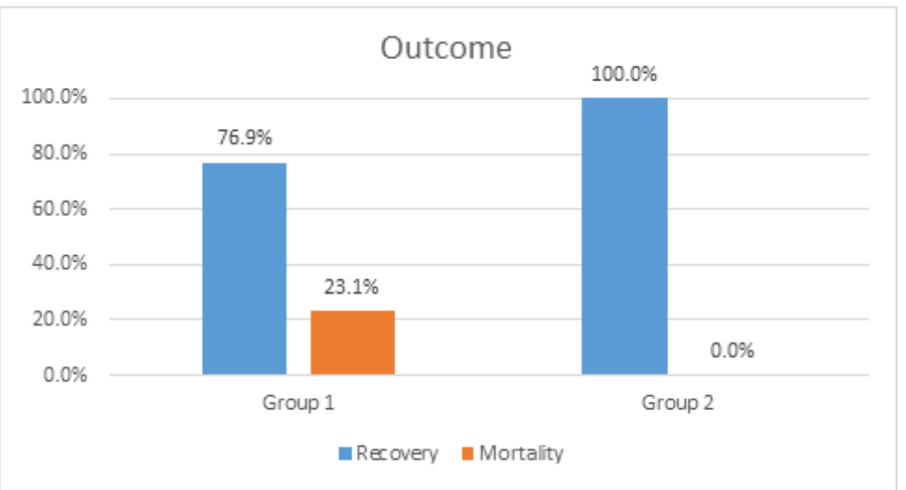

Fig. 2: Shows outcome between two studied groups, 38 (100%) of patients were recovered and no mortalities in group 2, while 21 (23.08%) died and 70 (76.9%) were recovered in group 1 after treatment, with significant increase of died patients between two groups in as *p-Value <0.05*.

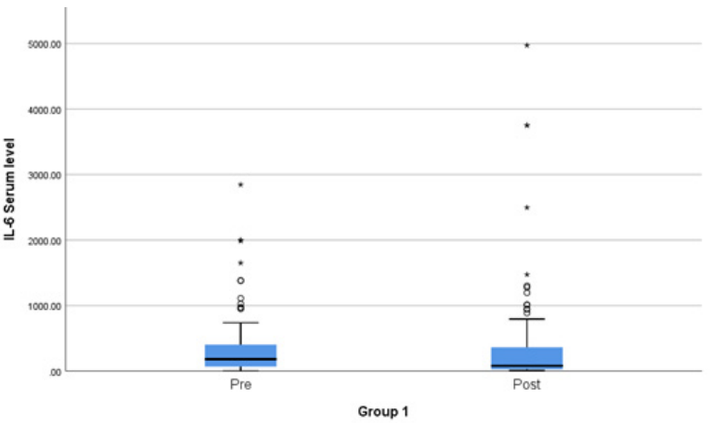

Fig. 3: Shows IL-6 serum level (pg/mL) pre and post tocilizumab injection as it is an indicator for cytokine storm in COVID-19 patients. Among group 1 median (IQR) of IL-6 serum level (pg/mL) was: Pre tocilizumab injection was 180 (66.5 – 405) and ranged from 1.5 to 2845, while post tocilizumab injection was 81.4 (27.6 – 363.7) ranged from 6.36 to 4972 with significant decrease between pre and post as *p-Value* was *0.01*.

Table (4): Shows relation between IL-6 serum level (pg/mL) and chest CT severity score within group 1.

Table 4: Change in IL-6 serum level pre and post tocilizumab injection within different severity scores of chest CT.

IL-6 serum level (pg/mL) Vs. Chest CT severity		Mild	Moderate	Severe
		Mean ± SD	Mean ± SD	Mean ± SD
Pre		161.6 ± 97.51	492.06 ± 71.21	322.61 ± 101.84
Post		135.32 ± 159.68	303.3 ± 116.61	845.08 ± 166.78
Pairwise comparisons	Mean difference ± SD	-26.28 ± 174.55	-188.77 ± 127.48	522.47 ± 182.32
% of Change		-16.3%	-38.4%	162%
p-Value		0.881	0.142	0.005

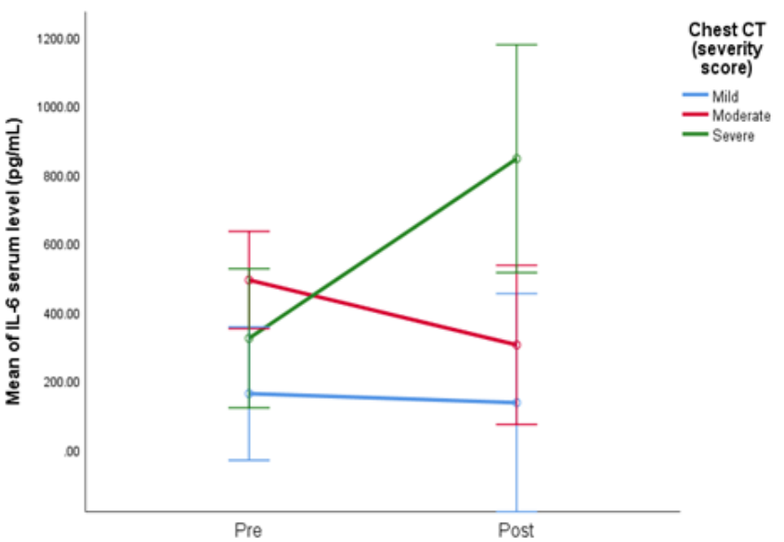

Fig. 4: Shows change in level IL-6 serum level (pg/mL) pre and post tocilizumab injection within different severity scores of chest CT, there was a decrease in the mild group by 16.3% and in moderate group by 38.4%. On the other hand a marked increase was observed in severe group by 162% with significant difference in the severe group only as *p-value* was *0.005*.

Table (5): Shows relation between IL-6 serum level (pg/mL) and outcome within group 1.

Table 5: Change in IL-6 serum level pre and post tocilizumab injection within outcome.

IL-6 serum level (pg/mL) Vs. outcome		Recovery	Mortality
		Mean ± SD	Mean ± SD
Pre		365.53 ± 59.21	358.64 ± 108.1
Post		123.89 ± 78.94	1276.93 ± 144.13
Pairwise comparisons	Mean difference ± SD	-241.64 ± 89.75	918.29 ± 163.86
% of Change		-66.1%	256%
p-Value		0.008	<0.001

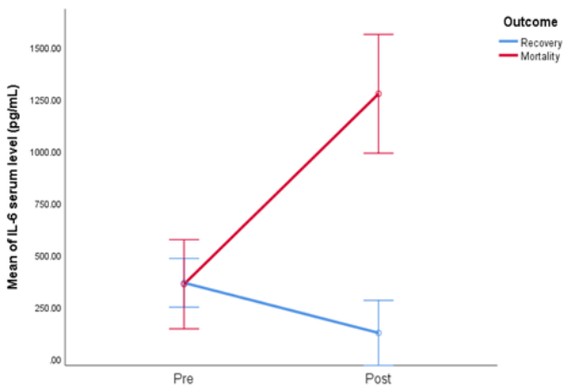

Fig. 5: Shows change in level IL-6 serum level (pg/mL) pre and post tocilizumab injection with outcome, there was a decrease in recovery group by 66.1%, while there was a marked increase in mortality group by 256% with significant difference within two groups as *p-value* was 0.008 and *<0.001* in both groups respectively.

Table (6): Shows the sensitivity and specificity of IL-6 serum level (pg/mL) within group 1 to assess its predictive role in mortality of cases, and found that the sensitivity of IL-6 was 90.5%, specificity was 85.7%, with AUC 0.919.

Table 6: Sensitivity and specificity of IL-6 after Tocilizumab injection to predict mortality.

AUC	Sig.	Cut-off value	Sensitivity	Specificity	+PV	-PV
0.919	<0.001	>220.1	90.48	85.71	65.5	96.8

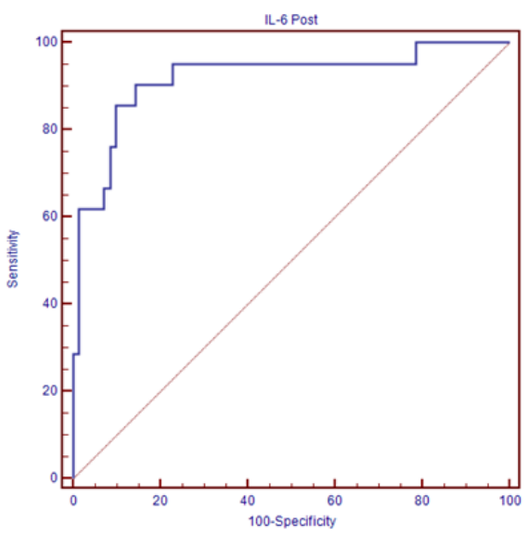

Fig. 6: Shows Roc curve of IL-6 serum level (pg/mL) post tocilizumab injection to differentiate the mortality from patients who discharged from the hospital within group 1.

DISCUSSION

In December 2019 in Wuhan, China, where SARS-Cov-2 was first discovered that cause COVID-19, and the pandemic was declared in 2020. It had been reported to cause injury to the respiratory tract and most patients developed pneumonia, which can worsen rapidly into respiratory failure[5].

In COVID-19 individuals, higher IL-6 serum levels were associated with an unfavourable prognosis and worsening clinical outcomes. It has been showed that IL6 levels above 80 pg/ml are sufficient for identifying COVID-19 patients at high risk of respiratory failure (10). IL-6R inhibitors suggested to be an active therapy for the treatment COVID-19 patients and might decrease disease complications and mortality[14].

Regarding comparison between the two studied groups, there was a significant difference between both groups in age as old patients presented in group 1 who received tocilizumab. Other studies, figured a significant difference between age and severity of the disease and it was higher in severe and pneumonic groups respectively[15, 16]. Similarly Wang *et al*., 2021, revealed that elderly age is a warning sign for the onset of ARDS in COVID-19 patients. This could be explained as the older patients had more severe symptoms and require intervention with tocilizumab[17].

There was a significant difference in the mortality rate between the two studied groups as the mortality rate was 23.1% in group 1, although they received tocilizumab and no mortalities in group 2, that may be because of difference between the two studied groups in clinical presentation, the deterioration and severity of symptoms and the decision of intervention with tocilizumab as it is related to not only the IL-6 serum level but also on the whole poor general condition of the patient.

In our study, the significance decrease in IL-6 might be in the low baseline patients, but the patients who had high IL-6 at baseline continue the increase even after treatment with tocilizumab. The final result within the group who received tocilizumab showed a decrease in IL-6 serum level after treatment. So Galván-Román *et al*., 2021 and many authors recommended further studies to assess the relation between low and high baseline IL-6 levels and their levels post tocilizumab injection[18].

In the same vessel, previous studies mentioned that IL-6 serum levels are not strongly related to the effect of the drug on patient as it may be temporarily increased after treatment with tocilizumab as this drug is IL-6 antagonist and blocks the action of IL-6 and does not interfere with the synthesis of IL-6 itself. However, it may successfully cure severe COVID-19 patients through inhibiting IL-6 inflammatory response[5].

We assessed the relation between IL-6 and CT chest severity within group 1, and it was demonstrated that there was a decrease in IL-6 serum level within both mild and moderate groups pre and post injection with tocilizumab by 16.3% and 38.4% respectively, while there was a significant increase in the patients who had severe CT findings by 162% pre and post injection although they received tocilizumab (Fig. 4). The aberrant and excessive immune cells in COVID-19 patients might affect the pulmonary circulation in high amounts and play a negative influence in the immune system leading to respiratory faliure and the rapid mortality[13]. Receiving tocilizumab could lower the risks in mild and moderate groups not in the severe group as if the cytokine storm was flaring up, IL-6 antagonists individually can not put it out.

In our result, the relation between IL-6 serum level and mortality was assessed within group 1, we concluded that there was a significant decrease in IL-6 serum level within recovered group by 66.1%, while significant increase in IL-6 serum level within mortality group by 256% pre and post injection with tocilizumab was present, which goes in line with Huang *et al*., 2020 and Wang *et al*., 2021, who observed that IL-6 serum level was higher in died group[17, 19]. Hermine *et al*., 2021, set up that tocilizumab might lower the 14-day but not one month mortality from hospitalization. So the series follow up of IL-6 serum level is crucial in deciding intervention timing of IL-6R inhibitors as elevated levels of specific cytokines such as IL-6 during the early stages of disease may contribute to multi organ damage[14].

Roc curve was done to find out to how far we can use IL-6 serum level post tocilizumab injection in the prediction of mortality and we found that the cut-off value was >220.1 by sensitivity 90.5%, specificity 85.7% and the AUC was 0.919, which means that the high serum levels of IL-6 more than 220.1 represent powerful marker to predict the increasing risk of mortality even if the patient had received tocilizumab. Our results go in alignment with Huang *et al*., 2020, who found that cytokine release syndrome predicts bad prognosis in COVID-19. In the same context, IL-6 serum level higher than a definite cut-off was a functional biomarker for catastrophic outcomes, the authors revealed that the cut-off value was 453.85 pg/ml by sensitivity, specificity 100% for both and AUC was 1.0. The difference in cut-off values could be clarified by the difference in sample sizes between two studies as their study was conducted on 29 patients only[19].

Moreover, Yang *et al*. 2020, They investigated 48 cytokines in the serum of COVID-19 patients, including IL-6, and observed that 14 of these cytokines were significantly elevated. IP-10, MCP-3, and IL-1ra have been found as indicators for illness severity and catastrophic outcomes among these 14 cytokines. So when we assess IL-6 serum level after intake of IL-6 antagonist, we should take into consideration other cytokines, as well as usage of combinations of many blockages for these cytokines may be needed to reveres their effect on the body and improve the mortality[20].

Finally, IL-6 serum level may be a major indicator but not the sole driver in the pathology of COVID-19, consequently also the early intervention and treatment can influence the benefit of IL-6 blockade therapy in control the deterioration of symptoms, alleviate MOD, improve the mortality rate and impact.

CONCLUSION AND RECOMMENDATIONS

The present study is considered as a preliminary step in the way to investigate the role of tocilizumab in lowering severity of disease and mortality of patients who diagnosed with SARS CoV2 infection. The results of this study focused on the role of using IL-6 antagonists such as tocilizumab to reduce the effect of body immune response 'hyperinflammtory immune response' against COVID-19 infection in reducing the mortality of severely ill patient if the patient received the treatment as soon as possible before had a high IL-6 serum levels. However, the results of the present study were may be affected by certain drawbacks, such as the small sample size and the short period of the study. In addition, lacking of some data at the patients' medical records. Further prospective studies are recommended to investigate the effect of tocilizumab in different populations and to how far it can reduce the severity and mortality in different countries. Further clinical trials are recommended to find out the relation between severity of the disease & mortality and cytokines other than IL-6. Broad scale studies are recommended on larger number of patients with different co-morbidities to assess if there a relation between co-morbidities and response to tocilizumab. Detailed tissue culture studies are needed synchronise with clinical trials to highlight the cytotoxic effect of COVID-19 on cell-line in vitro as well as its relevant behaviour with immune cells and mediators.

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CONFLICT OF INTEREST

There are no conflicts of interest.

REFERENCES

1. Rothan, H. A., & Byrareddy, S. N. (2020). The epidemiology and pathogenesis of coronavirus disease (COVID-19) outbreak. Journal of autoimmunity, 109, 102433. https://doi.org/10.1016/j.jaut.2020.102433.

2. Qin, C., Zhou, L., Hu, Z., Zhang, S., Yang, S., Tao, Y., Xie, C., Ma, K., Shang, K., Wang, W., & Tian, D. S. (2020). Dysregulation of Immune Response in Patients With Coronavirus 2019 (COVID-19) in Wuhan, China. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America, 71(15), 762–768. https://doi.org/10.1093/cid/ciaa248.

3. Li, G., Fan, Y., Lai, Y., Han, T., Li, Z., Zhou, P., Pan, P., Wang, W., Hu, D., Liu, X., Zhang, Q., & Wu, J. (2020). Coronavirus infections and immune responses. Journal of medical virology, 92(4), 424–432. https://doi.org/10.1002/jmv.25685.

4. Coperchini F., Chiovato L., Croce L., Magri F., & Rotondi M. (2020). The cytokine storm in COVID-19: An over view of the involvement of the chemokine/chemokine-receptor system. Cytokine and Growth Factor Reviews.

5. Xu, X., Han, M., Li, T., Sun, W., Wang, D., Fu, B., & Wei H. (2020). Effective treatment of severe COVID-19 patients with tocilizumab. Proceedings of the National Academy of Sciences of the United States of America,117(20), 10970–10975. https://doi.org/10.1073/pnas.2005615117.

6. Gubernatorova E., Gorshkova E., Polinova A., & Drutskaya M. (2020). IL-6: Relevance for immunopathology of SARS-CoV-2. Cytokine and Growth Factor Reviews. Elsevier Ltd.

7. Suresh, K., Figart, M., Formeck, S., Mehmood, T., Abdel Salam, M., & Bassilly, D. (2021). Tocilizumab for the Treatment of COVID-19-Induced Cytokine Storm and Acute Respiratory Distress Syndrome: A Case Series From a Rural Level 1 Trauma Center in Western Pennsylvania. Journal of investigative medicine high impact case reports, 9, 23247096211019557. https://doi.org/10.1177/23247096211019557.

8. Aziz M., Fatima R., & Assaly R. (2020). Elevated interleukin-6 and severe COVID-19: A meta-analysis. Journal of Medical Virology. John Wiley and Sons Inc.

9. Tanaka, T., Narazaki, M., & Kishimoto, T. (2018). Interleukin (IL-6) Immunotherapy. Cold Spring Harbor perspectives in biology, 10(8), a028456. https://doi.org/10.1101/cshperspect.a028456.

10. Herold, T., Jurinovic, V., Arnreich, C., Lipworth, B. J., Hellmuth, J. C., von Bergwelt-Baildon, M., Klein, M., & Weinberger, T. (2020). Elevated levels of IL-6 and CRP predict the need for mechanical ventilation in COVID-19. The Journal of allergy and clinical immunology, 146(1), 128–136.e4. https://doi.org/10.1016/j.jaci.2020.05.008.

11. Chen, X., Zhao, B., Qu, Y., Chen, Y., Xiong, J., Feng, Y., Men, D., Huang, Q., Liu, Y., Yang, B., Ding, J., & Li, F. (2020). Detectable Serum Severe Acute Respiratory Syndrome Coronavirus 2 Viral Load (RNAemia) Is Closely Correlated With Drastically Elevated Interleukin 6 Level in Critically Ill Patients With Coronavirus Disease 2019. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America, 71(8), 1937–1942. https://doi.org/10.1093/cid/ciaa449.

12. Abidi, E., El Nekidy, W. S., Alefishat, E., Rahman, N., Petroianu, G. A., El-Lababidi, R., & Mallat, J. (2022). Tocilizumab and COVID-19: Timing of Administration and Efficacy. Frontiers in pharmacology, 13, 825749. https://doi.org/10.3389/fphar.2022.825749.

13. Zhang, C., Wu, Z., Li, J., Zhao, H., & Wang, G. (2020). Cytokine release syndrome in severe COVID-19: interleukin-6 receptor antagonist. International Journal of Antimicrobial Agents, 55(2020), 105954–105960. https://doi.org/10.1016/j.ijantimicag.2020.105954.

14. Hermine, O., Mariette, X., Tharaux, P. L., Resche-Rigon, M., Porcher, R., Ravaud, P., & CORIMUNO-19 Collaborative Group (2021). Effect of Tocilizumab vs Usual Care in Adults Hospitalized With COVID-19 and Moderate or Severe Pneumonia: A Randomized Clinical Trial. JAMA internal medicine, 181(1), 32–40. https://doi.org/10.1001/jamainternmed.2020.6820.

15. Liu, Y., Tan, W., Chen, H., Zhu, Y., Wan, L., Jiang, K., Guo, Y., Tang, K., Xie, C., Yi, H., Kuang, Y., & Luo, Y. (2021). Dynamic changes in lymphocyte subsets and parallel cytokine levels in patients with severe and critical COVID-19. BMC infectious diseases, 21(1), 79. https://doi.org/10.1186/s12879-021-05792-7.

16. Yang, P. H., Ding, Y. B., Xu, Z., Pu, R., Li, P., Yan, J., Liu, J. L., Meng, F. P., Huang, L., Shi, L., Jiang, T. J., Qin, E. Q., Zhao, M., Zhang, D. W., Zhao, P., Yu, L. X., Wang, Z. H., Hong, Z. X., Xiao, Z. H., Xi, Q., … Cao, G. W. (2020). Increased circulating level of interleukin-6 and CD8+ T cell exhaustion are associated with progression of COVID-19. Infectious diseases of poverty, 9(1), 161. https://doi.org/10.1186/s40249-020-00780-6.

17. Wang, J., Yang, X., Li, Y., Huang, J. A., Jiang, J., & Su, N. (2021). Specific cytokines in the inflammatory cytokine storm of patients with COVID-19-associated acute respiratory distress syndrome and extrapulmonary multiple-organ dysfunction. Virology journal, 18(1), 117. https://doi.org/10.1186/s12985-021-01588-y.

18. Galván-Román, J. M., Rodríguez-García, S. C., Roy-Vallejo, E., Marcos-Jiménez, A., Sánchez-Alonso, S., Fernández-Díaz, C., Alcaraz-Serna, A., Mateu-Albero, T., Rodríguez-Cortes, P., Sánchez-Cerrillo, I., Esparcia, L., Martínez-Fleta, P., López-Sanz, C., Gabrie, L., Del Campo Guerola, L., Suárez-Fernández, C., Ancochea, J., Canabal, A., Albert, P., Rodríguez-Serrano, D. A., … REINMUN-COVID Group (2021). IL-6 serum levels predict severity and response to tocilizumab in COVID-19: An observational study. The Journal of allergy and clinical immunology, 147(1), 72–80.e8. https://doi.org/10.1016/j.jaci.2020.09.018.

19. Huang, L., Zhao, X., Qi, Y., Li, H., Ye, G., Liu, Y., Zhang, Y., & Gou, J. (2020). Sepsis-associated severe interleukin-6 storm in critical coronavirus disease 2019. Cellular & molecular immunology, 17(10), 1092–1094. https://doi.org/10.1038/s41423-020-00522-6.

20. Yang, Y., Shen, C., Li, J., Yang, M., Wang, F., Li, G., Li, Y., Xing, L., Peng, L., Wei, J., Cao, M., Zheng, H., Wu, W., Zou, R., Li, D., Xu, Z., Wang, H., Zhang, M., & Liu, Y. (2020). Exuberant elevation of IP-10, MCP-3 and IL-1ra during SARS-CoV-2 infection is associated with disease severity and fatal outcome. https://doi.org/10.1101/2020.03.02.20029975.