Journal of Regenerative Medicine

Research Article   A SCITECHNOL JOURNAL

Cytokine Storm Goes Hand in Hand with Prolonged Fever in SARS COVID-19: A Study on 1324 Patients Database of Past 1 Year

Vijay Bakhtar[1]*, **Niyati Bakhtar[1]**, **Nikhil Bakhtar[1]**, **Kirit Pandey[2]**, **Neha Bakhtar[3]**

Abstract

Background: Cytokine storm syndrome is a cascade of intensified immune responses disposing the immune system to exhaustion, which might eventually result in organ failure and fatal respiratory distress. Infection with severe acute respiratory syndrome-coronavirus-2 results in uncontrolled production of cytokines and eventually the development of cytokine storm syndrome. Clinical intervention in patients with SARS has demonstrated upregulation of cytokine production in patients with prolonged fever. Such an association between fever & cytokine storm is studied in the present research which is a hospital-based case-control study of COVID-19 patients. To the best of our knowledge, the present study is the first study of Indian population, which highlights the link between prolonged fever, Lymphocyte count and cytokine profile in patients with the COVID-19 disease.

Methods: We conducted a hospital-based case–control study of patients admitted for COVID-19 with prolonged fever and short duration of fever with a positive SARS-CoV-2 reverse transcriptase polymerase chain reaction (RT-PCR). All the patients underwent investigations which includes HRCT chest, Complete Blood Count (CBC), Renal and liver panel, C- Reactive Protein (CRP), Lactate dehydrogenase (LDH), Interleukin-6 (IL-6) and Ferritin, as a part of their COVID-19 protocol clinical laboratory findings at regular intervals till discharge.

Conclusion: The study reported that prolonged fever i.e. fever > 7 days from beginning of illness was associated with increased risk of cytokine storm from COVID-19 than patients with short duration of fever. There was also a marked reduction in Lymphocyte count with increased levels of inflammatory markers and fever, which is again considered an indicator of poor prognosis.

The study concludes that prolonged fever should be considered as one of the earliest markers for predisposing the patient to the cytokine storm. These early signs if diagnosed correctly right at the inception of complications the further progression of cytokine storm could be avoided. Which can minimize the mortality and foster the chances of the patient's recovery from this deadly disease.

Keywords:

COVID-19, Cytokines Storm, Prolonged Fever, Short Duration of Fever

*Corresponding author: Vijay Bakhtar, Department of Cardiologist, Director of Bakhtar Hospital, Amravati, Tel: 08668324946; E-mail: ashwini2niar@gmail.com

Introduction

SARS CoV-2 (severe acute respiratory syndrome) is a novel severe acute respiratory syndrome coronavirus. In Wuhan, China on December 31, (2019) was reported a cluster of viral pneumonia cases, subsequently identified as coronavirus disease 2019 (COVID-19). However, the newer strains and their associated signs and also complications emerging in COVID-19 are a continuing challenge to medical health systems all over the world and the scenario is still getting inferior. The COVID-19 poses an increasing threat to humans with a fatality rate of 6.4 % so far. Most commonly COVID-19 reports dry cough symptoms along with acute respiratory illness, with high variations in fever fatigue. Particularly, about 72% - 98.6% of a patient's shows prolong fever. Prolong fever, i.e. fever > 7 days has shown to be a detrimental sign in many viral infections including dengue. Similarly, fever of longer duration and illness extending in the second week of COVID-19 disease is also expected to worsen the outcomes. Cytokines are produced by several immune cells, including the innate macrophages, dendritic cells, natural killer cells and the adaptive T and B lymphocytes. Infection of COVID-19 is accompanied by an inflammatory response with the release of a large amount of pro-inflammatory cytokines in an event known as "*cytokine storm*". The host cell immune response to the SARS-CoV-2 virus is hyperactive resulting in an extreme inflammatory reaction. Some studies evaluating cytokine profiles from COVID-19 patients have shown that the cytokine storm is directly related with lung injury which ultimately results in multi-organ failure [1,2].

Evidences show that complications of COVID-19 occur in 2nd week of illness and their significance of variation in fever with regards to the development of complications is still unknown [3]. The immune system has a delicate mechanism capable of responding to various pathogenic infections.

In this study, we have examined the characteristics of patients who developed prolonged fever and studied its association to cytokine levels and lymphocyte count. Our aim is to study the basic, clinical parameter of the fever as an early marker to sense the risk of forthcoming cytokine storms [4]. Which if controlled at the inception itself, can reduce the COVID-19 associated mortality and increase the chances of the patient's recovery.

Methodology

Data collection and clinical management

We conducted a hospital-based case–control study of patients, who were admitted to '*Tertiary health care center*' Amravati, Maharashtra (India). With a positive SARS-CoV-2 reverse transcriptase polymerase chain reaction (RT-PCR) assays from date 01-August-2020 to 03-July-2021.

Upon admission, all patients underwent a HRCT chest score, Complete Blood Count (CBC). Renal and liver panel, C-Reactive Protein (CRP), Lactate dehydrogenase (LDH), Interleukin -6 (IL-6), Ferritin, through clinical laboratory findings at regular intervals till discharge [5,6].

SARS-CoV-2 PCR, patients who tested positive were not discharged until they had 2 negative RT-PCR test, 24 hours apart it's

a hospital-based protocol, which is based on literature. Standardized template was used for recording daily signs and symptoms, vital signs and treatment offered. Serial CBCs and HRCT chest score were done for those with prolonged fever and the records were collected. For cases with prolonged fever, examinations were repeated at a point of fever recurrence [7].

Definitions and Outcomes

Fever was defined as a temperature of ≥ 38 ∘ C or higher. Duration of fever was calculated from the date of 1st symptoms onset to the date of defervescence. During the course of hospital treatment, cases with prolonged fever were those showing fever ≥ 7 days. Cases with Saddleback fever were defined as patients with recurrence of fever lasting < 24 h, after defervescence, beyond day 7 of illness [8]. Cases without prolonged fever were considered as control cases. The patients who did not show any symptoms of fever are excluded from the analysis.

Lymphopenia is defined as lower Lymphocyte count than the normal range (20-40%).

Statistical Analysis

The Mann-Whitney U test was used to evaluate the difference in medians of continuous variation. For cytokine profile, Mann-Whitney U test was applied to find out the significance difference in fever pattern. Statistical analyses were performed in MS Excel. Regression analysis was used to determine the statistical significance of variables to find out dependency of lymphocyte on cytokine profile. i.e., LDH, CRP, IL-6, and Ferritin. The P value of <0.05 indicated statistical significance [9].

Results

We screened total 1324 patients admitted to 'Tertiary health care center' Amravati, Maharashtra (India) from 01 August 2020 to 03 July 2021.

From these studies, total 156 patients are excluded because of the no. of patients, i.e. 106/156 patients founded with no prolonged fever but having same common symptoms. And remaining 50 patients excluded because of they were found with no short duration of fever and asymptomatic [10]. Data was collected from the remaining 1168 (88.21%) as a cohort control group. Here, we divided control data into a prolonged fever/recurrent fever included with 315 (23.79%) patients in Group-A, and in Group-B included with 853 (64.27%)

patients with short duration of fever; of all study subjects were male, 37.73% (475/1324) of all subject were female and median age [Interquartile range (IQR)] was 52 (17-92) years (Table 1).

Since the Confidence Interval (CI) of (0.8858 to 1.8200) spans 1.0 to increase Odd Ratio (OR) 1.27 of patients with fever having symptomatic and asymptomatic in COVID-19 was slightly higher odd in patients with no fever having symptomatic and asymptomatic [OR:1.27, 95%: CI (0.8858-1.82)] and was statistically significant (p=0.0001) [11].

Demographics were similar across 2 categories, i.e. prolonged fever and short duration of fever. Group-A presents the data of prolonged fever more than or equal to 7 days and Group-B shows the short duration of fever i.e. fever less than 7 days. Patients having prolonged fever had a median duration of fever, which last for approximate 7-10 days. The patients with prolonged fever show significantly associated with C-Reactive Protein, Lactate dehydrogenase, Interleukin-6, and Ferritin, on the other hand the Lymphocyte count was also significantly related with short duration of fever (Table 2).

In this present study age is not a significant factor in a cytokine storm with prolonged fever and short duration of fever. The cases with prolonged fever had LDH 305 u/l (88-978) v/s the short duration of fever 230 u/l (88-978; p <.01) it indicates rises to LDH level and its significance associated with short duration of fever. On the other hand, the cases with prolonged fever had more levels of CRP 20 mg/l (1.40-192) compared to the short duration of fever were found 13 mg/l (1.42-175; p<.01). The cases with prolonged fever were Ferritin found to be around 247 ng/ml (7-912) and in a short duration of fever were 195 mg/ml (5-982; p<.01). The cytokine and chemokine concentrations from a prolonged fever were found to have higher levels of inflammatory IL-6 7.8 pg/ml (0.20-517) and in short duration of fever 5.8 pg/ml (0.12-444.9) p<.01]. The average of Lymphocyte count in prolonged fever patients was around 16% (3-79) and in a short duration of fever was found to be 21% (4-80; p<.01) it shows a significant difference in prolonged fever cases. All the Group-A patients showed significantly [12,13] higher levels of the inflammatory parameters tested, which were C-Reactive Protein, Lactate dehydrogenase, Interleukin-6, and Ferritin is significantly associated as compared to the short duration of fever. Besides this the low Lymphocyte count was found in prolonged fever patients.

The patients with COVID-19 have high cytokine levels, which can be considered as potential biomarkers for disease progression. The specific immune profile of COVID-19 can further induce infection and multiple organ dysfunction.

Table 1: Both an odds ratio (OR) and a relative risk (RR) are calculated from values in a 2 x 2 contingency table. A, B, C and D represent the number of subjects that fall into each cell.

	Symptomatic	Asymptomatic	Odds Ratio (OR)
Fever	853 (a)	315 (b)	853/315
No Fever	106 (c)	50 (d)	106/50
Total	959	365	2.70/2.12=**1.27**

Table 2: Demographic and Clinical laboratory features of Prolonged and Short duration of fever in COVID-19.

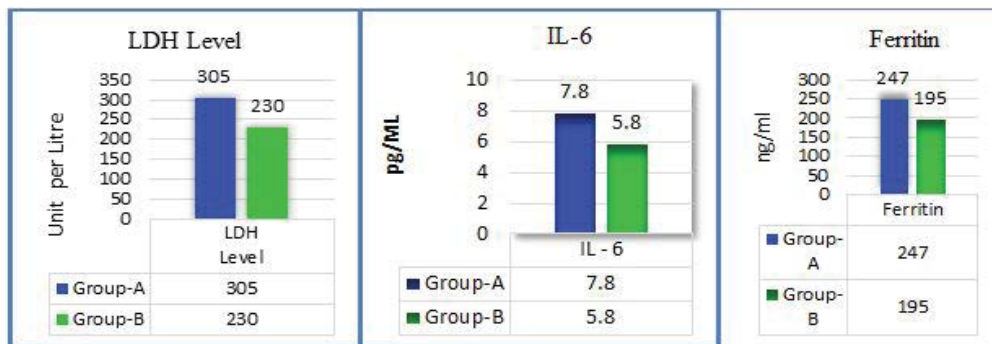|	Prolonged fever(n=315), No. (%) or median (IQR)	Short duration of fever(n=853), No. (%) or median (IQR)	P-Value
Age	52 (21-83)	52 (17-92)	0.2
LDH	305 (88-978)	230 (88-978)	<.01
CRP	20 (1.40-192)	13 (1.42-175)	<.01
Ferritin	247 (7-912)	195 (5-982)	<.01
IL-6	7.8 (0.20-517)	5.8 (0.12-444.9)	<.01
Lymphocytes	16 (3-79)	21 (4-80)	<.01

Figure 1: The graphical representation indicates the comparison of cytokines levels in COVID-19 infection.
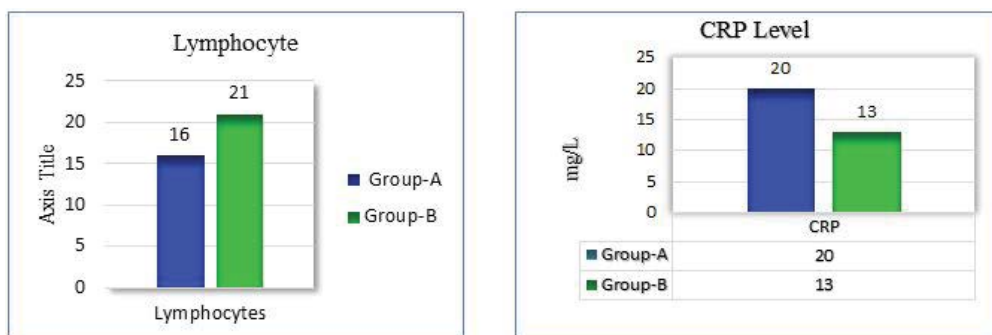

Figure 2: The graphical representation indicates the comparison of lymphocyte and CRP levels in COVID-19 infection.

From the following graphical observational findings (Figure 1) we can say that, fever duration impacts the inflammatory response which leads to the occurrence of a cytokine storm. The inflammatory cytokine included LDH, Ferritin, IL-6 were found higher in prolonged fever as compared with short duration of fever.

The color of graphical representation indicates a blue color for prolonged fever i.e. (Group-A) and green for the short duration of fever i.e. (Group-B). The exaggerated inflammatory response with a large amount of pro-inflammatory cytokine markers the tissue injury and more pulmonary infection (Figure 1).

Correlation Between Fever Pattern and Cytokine Profile

To investigate whether the cytokine profile is correlated with the fever pattern. In prolonged fever (Group-A) cases we found that Lymphocytes were significantly dependent on CRP, i.e. with increased levels of CRP there was a decrease in Lymphocyte count viz Lymphopenia. On the other hand, other cytokines LDH, Ferritin and IL-6 not were not significantly affecting the Lymphocyte count (Figure 2). A color of graphical representation indicates, a blue color for prolonged fever i.e. (Group-A) and green for the short duration of fever, i.e. (Group-B).

The above graphical representation indicated that CRP level in prolonged fever is significantly dependant on Lymphocyte count. Moreover, the average lymphocyte count was observed to be 16% in group A patients and 21% in group B patients which denotes significant lower levels of Lymphocytes in patients with prolonged fever compared to the short duration of fever. Thus, the study founds LDH, CRP, Ferritin, IL-6 in high levels of inflammatory cytokines and the low Lymphocyte count were significantly associated with prolonged fever.

Discussion

In this study, we have presented, the association between the duration of fever and cytokine levels in COVID-19 disease. This retrospective study was conducted on the data 1324 hospitalized patients, the laboratory findings of the patients include higher levels of CRP, LDH, Ferritin, IL-6, and lower levels of Lymphocyte count cases. Apart from the usual symptoms the study cases also reported some not so common symptoms like, Dyspnea, Anorexia, Hypotension and some other common symptoms are the cough or shortness in breathing present in (12%) of the cohort control study. The fever pattern experienced by patients are due to the person-to-person differences in their immune response, (30%) of patients are evaluated with Malaise, as well as (8%) of patients were seen with vomiting and diarrhoea [14]. The immune illness of the patient showed different signs and symptoms (18%) of them evaluated with loss of appetite and (16%) of patients was examined with loss of smell and in loss of taste. Inflammation of mucous membrane of the nose caused by viral infection or the Rhinitis significant with 5% of patients, and the other remaining (28%) of patients having general weakness. Apart from the above symptoms 3 patients were even present with Pancreatitis.

The mechanism of cytokine upregulation in case of prolonged fever can be explained as below:

With prolonged fever during COVID-19, an exacerbated pulmonary and systemic inflammatory response occur with increased

levels of inflammatory markers which are cytokines and chemokines such as CRP, LDH, Ferritin, IL-6 etc. all of which results in cytokine storm. This is a form of excessive host response to pathogen which ultimately leads to reduced WBCs especially affecting lymphocytes. This results in decreased levels of lymphocytes and cause the lymphopenia. The severity of SARS-CoV-2, is also related to the T-cell dysfunctionality [15]. It has been observed that SARS-CoV-2 induced cytokines may damage the capacity of T-cells.

The present study also evidenced similar findings in their patients. Our findings are in concordance with a landmark study done by [16], who found that, prolonged ill patients had high attentiveness of pro-inflammatory cytokines, such as interleukin (IL)-6, compared to those who are normally ill patients. The high level of cytokines profile indicated a poor diagnosis in COVID-19. Besides, this pro - inflammatory cells, mainly involving macrophages and T-helper cells, has been found in lung tissues of patients with COVID-19 by post-mortem examination.

Another study done by [17], also reported that, patients with prolonged fever and the patients with normal fever at the early acute phase of illness of disease showed different immunological responses. The differences in cytokine & chemokine profile were seen between control patients with fever ≤ 7 days, the patient with prolonged fever and patients with saddleback fever at the early acute phase of illness. This corroborated well with our findings of increased levels of cytokine profile with COVID-19 infection during prolonged fever and normal fever.

Few other studies, including studies done by [18] have also shown the role of cytokines contributing to severe disease and poorer prognosis in COVID patients. Their study also showed similar results on lymphopenia and increased cytokine levels as evidenced by our study.

Along with Lymphopenia there is a significant difference in IL-6 and Ferritin levels between the prolonged fever and control patients in the present paper. The higher levels of CRP, LDH, IL-6, and Ferritin have been reported to be associated with increased viral load, lung injury, ICU admission and mortality.

Conclusion

The present study establishes the link between prolonged fever and cytokine storm in COVID-19 patients. It is observed that prolonged fever is the earliest sign to hint at the cytokine upregulation, which will further lead to cytokine storm. Hence, if the treatment with steroids and immunomodulators including Tocilizumab is offered at the advent of prolonged fever itself then, the further complications of cytokine storm could be avoided and thus mortality rate will be controlled. So, we can control cytokine storm.

References

1. Pung R, Chiew C, Young BE (2020) Investigation of three clusters of COVID-19 in Singapore: implications for surveillance and response measures. Lancet 395:1039-46.

2. WHO Coronavirus disease (2019) (COVID-19) Situation Report 127.

3. Wang D, Hu B, Hu C (2020) Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus-infected pneumonia in Wuhan, China. JAMA 32(11): 1061-1069.

4. Guan WJ, Ni ZY, Hu Y (2020) Clinical characteristics of coronavirus disease 2019 in China. N Engl J Med 382: 1708-1720.

5. Young BE, Ong SWX, Kalimuddin S (2020) Epidemiologic features and clinical course of patients infected with SARS-CoV-2 in Singapore. JAMA 323:1488-1494.

6. Qiu H, Wu J, Hong L (2020) Clinical and epidemiological features of 36 children with coronavirus disease 2019 (COVID-19) in Zhejiang, China: an observational cohort study. Lancet Infect Dis, 20: 689-696.

7. McCormack S, Dunn D, Desai M (2016) Pre-exposure prophylaxis to prevent the acquisition of HIV-1 infection (PROUD): effectiveness results from the pilot phase of a pragmatic open-label randomised trial. Lancet 387: 53-60.

8. Ragab D, Eldin HS, Taeimah M (2020) The covid-19 cytokine storm; What we know so far. front Immunol, 11:1446.

9. Huang C, Wang Y, Li X (2020) Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet 395: 497-506.

10. Ruan Q, Yang K, Wang W (2020) Clinical predators of mortality due to covid 19 based on analysis of data of 150 patients from Wuhan. Wuhan , China. Intensive Care Med 46: 846-848.

11. Sun D, Li H, Lu X (2020) Clinical feature of severe pediatric patient with coronavirus disease 2019 in Wuhan a single centers observational study. Wuhan, China. World J Pediatr 19: 1-9.

12. Tay JY, Lim P, Marimuthu K (2020) De-isolating COVID-19 suspect cases: a continuing challenge. Clin Infect Dis 71: 883-884.

13. Wu C, Chen X, Cai Y (2020) Risk factors associated with acute respiratory distress syndrome and death in patients with coronavirus disease 2019 pneumonia in Wuhan, China. JAMA Intern Med 180: 1-11.

14. Shi S, Qin M, Shen B (2020) Association of cardiac injury with mortality in hospitalized patients with COVID-19 in Wuhan, China. JAMA Cardiol 5: 802-810.

15. Zhe Xu, Shi L, Wang FS (2020) Pathological findings of COVID-19 associated with acute respiratory distress syndrome. Lancet Respir Med 8(4): 26.

16. Tang Y, Liu J, Zhang D (2020) Cytokine Storm in COVID - 19: The Current Evidence and Treatment Strategies. Front Immunol 10: 3389.

17. Deborah H, Choy CY, Chan YC (2020) Fever Patterns, Cytokine Profiles, and Outcomes in COVID-19. OFID 1-7.

18. Singhal T (2020) A Review of Coronavirus Disease-2019 (COVID-19). The Indian J Paediatrics 87(4): 281-286.

Author Affiliations

Top

¹Department of Cardiologist, Bakhtar Hospital, Amravati, India

²Department of Gynaecology and Obstetrics, Arm Forces Medical College, Pune, India

³Department of Senior Medical Officer, Army Medical Corps, MH Pulgaon, India