Original Research Article

Cytokine Interleukin-6 as an Inflammatory Biomarker in Patients Infected With COVID-19

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Conflict of interest: Nil

Abstract:

Background: The ongoing worldwide Corona virus Disease 2019 (COVID-19) pandemic has posed a huge threat to global public health. Numerous inflammatory markers are elevated in patients with severe disease relative to patients with milder conditions. Interleukin-6 (IL-6) was identified as potential inflammatory biomarkers in Covid-19 pandemic. IL-6 is a cytokine featuring pleiotropic activity. It induces synthesis of acute phase proteins such as C-reactive protein, serum amyloid A, fibrinogen and hepcidin in hepatocytes. Interleukin-6 is an early biomarker of infection and inflammation. IL-6 is one of the main mediators of inflammatory and immune response initiated by infection and injury in lungs.

Aims: To correlate serum IL-6 level in covid-19 patients and its relationship with severity of the disease.

Methodology: The study was conducted on three groups mild (N=30), moderate (N=30) and severe (N=30) covid-19 positive patients. Age matched covid-19 negative as controls (N=30) also recruited for this study. Serum IL-6 levels in covid-19 patients were measured by using an immune-enzymatic chemiluminescence assay.

Results: We found that serum IL-6 levels were significantly increased in covid-19 patient as compared to normal healthy controls. Our result shows that serum IL-6 levels were increased as disease progress from mild to severe condition of Covid-19 patients.

Conclusions: Serum IL-6 increased in COVID-19 due to enhanced cell mediate immune response, infection and tissue damage in lungs. Our results suggest that monitoring inflammatory markers may serve as an early warning system for progression to severe COVID-19.

Introduction

The worldwide Corona virus Disease 2019 (COVID-19) pandemic has posed a huge threat to global public health. Our country also faced this pandemic in crucial condition with large number of morbidity and mortality. The pathogen has been identified as a novel single stranded ribonucleic acid (RNA) beta corona virus named as severe acute respiratory syndrome corona virus 2 (SARS-CoV-2), which shares an approximately 80% similarity at nucleotide level with severe acute respiratory syndrome corona virus. COVID-19 represents a spectrum of clinical severity ranged from asymptomatic to critical pneumonia, acute respiratory distress syndrome (ARDS) and even death. Therefore, full monitoring of severe infected COVID-19 patients and effective early intervention are the fundamental measures for reducing mortality. [1]

Most of the evidence has suggested that inflammatory responses play a critical role in the progression of COVID-19. Inflammatory responses triggered by rapid viral replication of SARS-CoV-2 and cellular destruction can recruit macrophages and monocytes and induce the release of cytokines and chemokines. These cytokines and chemokines then attract immune cells and activate immune responses, leading to cytokine storms and aggravations. Several inflammatory markers have some tracing and detecting accuracy for disease severity and fatality. Inflammatory markers such as procalcitonin (PCT), serum ferritin, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) and interleukin-6 (IL-6) have been reported to be significantly associated with the high risks of the development of severe COVID-19. Moreover, increased levels of serum IL-6 are shown to be involved in COVID-19 pathogenesis and may serve as a potential biomarker for monitoring disease progression. [2]

The outbreak of corona virus disease (COVID-19) in December 2019, caused by the severe acute respiratory syndrome corona virus 2 (SARS-CoV-2). It poses a severe threat to global public health. Some studies suggest that numerous inflammation markers are elevated in patients in the intensive care unit or patients with severe disease relative to patients with milder conditions. [3]

Some evidence has suggested that inflammatory responses play a critical role in the progression of COVID-19. [4] Inflammatory responses triggered by rapid viral replication of SARS-CoV-2 and cellular destruction can recruit macrophages and monocytes and induce the release of cytokines and chemokines. These cytokines and chemokines then attract immune cells and activate immune responses, leading to cytokine storms and aggravations. Several inflammatory markers have some tracing and detecting accuracy for disease severity and fatality. IL-6 is a pleiotropic cytokine with a wide range of function. IL-6 produced from a single gene encoding a product of 212 amino acids, which is cleaved at the N terminus to produce a 184 amino acids peptide with a molecular weight between 22-27 kDa. [5]

IL-6 production is rapidly induced in the course of acute inflammatory response associated with injury, trauma, stress, infection, brain death, neoplasia, and other conditions. IL-6 is produced by fibroblast, activated T cell, activated monocytes or macrophages and endothelial cells. IL-6 is a pro-inflammatory cytokines, that secreted into the serum and induce a transcriptional inflammatory response through interleukin-6 receptor, alpha. IL-6 is a non specific marker and hence is not diagnostic for any specific disease. IL-6 was proposed to be associated with the severity of corona virus disease. [6]

The present study was proposed to correlate the serum levels of IL-6 with severity of COVID-19 and monitoring of the disease.

Methodology

The study was initiated after obtaining approval from the Institutional Ethics Committee, SRG Hospital & Jhalawar Medical College, Jhalawar Rajasthan. This study was carried as a retrospective study between July 2023 and December 2023. Nasopharyngeal swab was taken as sample for RT-PCR. This study was conducted first time in *Hadoti* region of Jhalawar, Rajasthan. In this study, we recruited total 90 cases and divided patients into three groups common (N=30), severe (N=30) and critical (N=30) cases of Covid-19 patients. The diagnosis and severity classification was based on Clinical Guidance for management of adult Covid-19 patients (Ministry of Health & Family welfare) and WHO interim guideline.

Classification of cases were as follow

A. Common cases: Covid-19 patients showing Fever, dry cough, nasal congestion, sore throat, lost sense of smell or taste, conjunctivitis, mild fatigue and imaging showed pneumonia.
B. Severe Cases: Adult cases meeting any of the following criteria: Respiratory distress ($\geq$ 30 breath/Min.), Oxygen saturation $\leq$93% at rest, Arterial partial pressure of Oxygen (PaO2)/ Fraction of inspired oxygen (FiO2) $\leq$ 300mmHg and Chest imaging that show obvious lesion progression within 24 hours.
C. Critical Cases: cases meeting any of the following criteria, Respiratory failure and requiring mechanical ventilation, shock, with other organ failure that requires ICU care.

Diagnosis of cases

Etiological: A positive RT-PCR detection of 2019-n CoV Nucleic acid. Quantitative estimation of IL-6 is done by fully automated, chemiluminescent immunoassay in serum.

Biostatistics: Descriptive statistics were applied to summarize data. Results were reported as mean with standard deviation. One-way ANNOVA was applied to detect significant difference among stratifications. Statistical Package for Social Science (SPSS) 25.0 software was used for statistical analysis and illustration.

Results

The mean concentration of IL-6 was 2.36±0.64 pg/ml, 5.84±1.12 pg/ml, and 21.21±6.57 pg/ml for the common, severe, and critical groups respectively ($P < 0.001$). Our result shows that serum IL-6 levels increased as disease progress toward critical condition.

Covid-19 Groups	Numbers	Serum IL-6 (Mean±S.D.)	p-Value	F-Value
Normal	30	2.36±0.64	<0.0001	
Severe	30	5.84±1.12	<0.0001	201.85
Critical	30	21.21±6.57	<0.0001	

Distribution of IL-6 Pg/ml according to groups
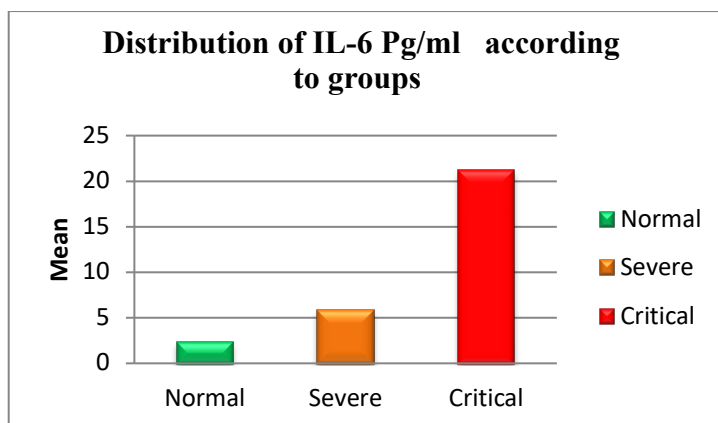
Discussion

We evaluated the association of serum biomarkers IL-6 in 90 patients diagnosed with COVID-19 infection. [7,8] Serum IL-6 concentration was tested and analyzed in all groups of Covid-19 patients. We obtain consistently high level of IL-6 in severe and critical group of patients as compare to normal group of covid-19 patients. We observed that serum IL-6 levels were significantly higher in patients with critically illness admitted in ICU compared with patients with severe and normal illness. [9,10]

Variable biomarkers were identified to predict the outcomes and severity of COVID-19 infections. Pan ji *et al* (2020) conducted a meta-analysis and found the association between inflammatory markers and severity of covid-19 patients. [11-14] They observed the elevated levels of serum interleukins-6 (IL-6) in severe covid-19 patients. Furong Jeng *et al* (2020) reported the associations of inflammatory markers with the severity of COVID-19. [15,16] Their meta-analysis highlights the association of inflammatory markers like Interleukin-6 (IL-6) with the severity of COVID-19. [17] Measurement of inflammatory markers might assist clinicians to monitor and evaluate the severity and prognosis of COVID-19. [18]

We also got a similar observation in our study. We also observed a positive correlation of serum levels of IL-6 with the severity of disease. [19]

Zing Jhang *et al* (2020) conducted a retrospective single-institutional study of 901 consecutive confirmed cases of covid-19. Serum IL-6 concentrations were tested on admission and/or during hospital stay. Serum IL-6 should be included in diagnostic work-up to stratify disease severity. They found elevated levels of IL-6 in covid-19 patients and also confirmed the finding that serum IL-6 should be included in diagnostic work-up to stratify disease severity. [20] It is recognize that excessive, multifunctional host immune response may play an important role in development and maintenance of critical stage of covid-19. [21,22]

Conclusion

Serum IL-6 should be included in diagnostic work-up to stratify disease severity. Therefore, full monitoring the severity of COVID-19 and effective early intervention are the fundamental measures for reducing mortality. Our research work will be helpful in making novel strategies for diagnosis, treatment and prognosis of Covid-19 patients. This study may be helpful to reduce mortality due to covid-19.

References

1. Worldometer. COVID-19 coronavirus pandemic updated January 19, 2021. https://www.0020worldometers.info/coronavirus
2. Jones SA, Novick D, Horiuchi S, Yamamoto N, Szalai AJ, Fuller GM. C-reactive protein: a physiological activator of interleukin 6.
3. Qiu H, Wu J, Hong L, Luo Y, Song Q, Chen D. Clinical and epidemiological features of 36 children with coronavirus disease 2019 (COVID-19) in Zhejiang, China: an observational cohort study. Lancet Infect. Dis. 2020; 20: 68 9–696.
4. Wang Z, Yang B, Li Q, Wen L, Zhang R. Clinical features of 69 cases with coronavirus disease 2019 in Wuhan, China. Clin. Infect. Dis. 2020; 71: 769–777.
5. Chen G, Wu D, Guo W et al. Clinical and immunological features of severe and moderate coronavirus disease 2019. J. Clin. Invest. 2020; 130: 26002020–2629.
6. Zheng Y, Xu H, Yang M et al. Epidemiological characteristics and clinical features of 32 critical and 67 noncritical cases of COVID-19 in Chengdu. J. Clin. Virol. 2020; 127: 104366.
7. Gao Y, Li T, Han M et al. Diagnostic utility of clinical laboratory data determinations for patients with the severe COVID-19. J. Med. Virol. 2020; 92: 791–796.
8. Veronese N, Demurtas J, Yang L et al. Use of corticosteroids in coronavirus disease 2019 pneumonia: a systematic review of the literature. Front. Med. (Lausanne). 2020; 7: 170.

9. Kermali M, Khalsa RK, Pillai K, Ismail Z, Harky A. The role of biomarkers in diagnosis of COVID-19 - a systematic review. Life Sci. 2020; 254:117788.

10. Malik P, Patel U, Mehta D et al. Biomarkers and outcomes of COVID-19 hospitalisations: systematic review and meta-analysis. BMJ

11. Velavan TP, Meyer CG. Mild versus severe COVID-19: laboratory markers. Int. J. Infect. Dis. 2020; 95: 304–307.

12. Guan WJ, Ni ZY, Hu Y et al. China Medical Treatment Expert Group for Covid-19. Clinical characteristics of coronavirus disease 2019 in China. N. Engl. J. Med. 2020; 382: 1708–1720.

13. Li LQ, Huang T, Wang YQ et al. COVID-19 patients' clinical characteristics, discharge rate, and fatality rate of meta-analysis. J. Med. Virol. 2020; 92: 577–583.

14. Quartuccio L, Semerano L, Benucci M, Boissier MC, De Vita S. Urgent avenues in the treatment of COVID-19: targeting downstream inflammation to prevent catastrophic syndrome. Joint Bone Spine. 2020; 87: 191–193.

15. Mo P, Xing Y, Xiao Y et al. Clinical characteristics of refractory COVID-19 pneumonia in Wuhan, China. Clin. Infect. Dis. 2020.

16. McGonagle D, Sharif K, O'Regan A, Bridgewood C. The role of cytokines including Interleukin-6 in COVID-19 induced pneumonia and macrophage activation syndrome-like disease. Autoimmun. Rev. 2020; 19: 102537.

17. 19. Zhang J, Hao Y, OuWet al. Serum interleukin-6 is an indicator for severity in 901 patients with SARS-CoV-2 infection: a cohort study. J. Transl. Med. 2020;18: 406.

18. Mehta P, McAuley DF, Brown M, Sanchez E, Tattersall RS, Manson JJ. HLH across speciality collaboration, UK. COVID-19: consider cytokine storm syndromes and immunosuppression. Lancet. 2020; 395: 1033–1034.

19. Benucci M, Damiani A, Infantino M, Manfredi M, Quartuccio L. Old and new antirheumatic drugs for the treatment of COVID-19. Joint Bone Spine. 2020; 87: 195–197.

20. Ji D, Zhang D, Chen Z et al. Clinical characteristics predicting progression of COVID-19. Lancet. 2020.

21. 23. G´omez-Pastora J, Weigand M, Kim J et al. Hyperferritinemia in critically ill COVID-19 patients – is ferritin the product of inflammation or a pathogenic mediator Clin. Chim. Acta. 2020; 509: 249–251.

22. Grang´e S, Buchonnet G, Besnier E et al. The use of ferritin to identify critically ill patients with secondary hemophagocytic lymphohistiocytosis. Crit. Care Med. 2016; 44(11): e1045–e1053.