ISSN: 0975-1556

Available online on www.ijpcr.com

International Journal of Pharmaceutical and Clinical Research 2021; 13(5); 298-304

Original Research Article

Interleukin-6 and Ferritin Levels and their Clinical Correlation in COVID-19 Patients: A Quantitative Assessment

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Received: 10-05-2021 / Revised: 04-06-2021 / Accepted: 14-08-2021

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Conflict of interest: Nil

Abstract

Aim: Quantitative Assessment of Interleukin-6 and Ferritin Levels and its Clinical Correlation among COVID-19 Patients. **Methods:** The cross-sectional analytical study was conducted in the Department of Pathology, Darbhanga Medical College and Hospital, Darbhanga, Bihar, India for 1 year after taking the approval of the protocol review committee and Institutional Ethics Committee. 120 COVID-19 positive patients, diagnosed upon admission by RT-PCR of oropharyngeal swabs with or without respiratory symptoms were included in the study group. **Results:** Group A had 55 patients among which 7 patients were asymptomatic and 48 had mild symptoms with RR <24/m and SpO2 >94% in room air, the number of patients with co-morbidities like type 2 diabetes mellitus were 15, with hypertension and cardiac disorders were 10 and with respiratory disorders were 5 in Group A, Group B there were 30 patients among which 9 had mild symptoms and 21 patients had moderate symptoms with RR: 24-30/m (or) SpO2: 90%-94% at room air. The mean levels with standard deviation of Serum IL-6 and serum ferritin in Group A, Group B and Group C patients, respectively has depicted in. table 2. On pairwise comparison by Mann-Whitney U test among the groups it shows that the mean IL-6 levels are significantly different in all the three groups. Kruskal Wallis pairwise comparison shows IL-6 levels to be significantly increased in Group C (35) patients with severe disease compared to Group A and Group B patients with mild and moderate disease respectively (p-value <0.001). Serum Ferritin levels did not show any statistically significant difference among Group A, Group B and Group C; however, ferritin levels were markedly increased in Group C patients with severe disease (p-value=0.44). **Conclusion:** Serum IL-6 levels independently showed a good correlation with disease severity among COVID-19 patients, and serum ferritin levels was elevated only in severely symptomatic individuals with COVID-19 infections. Hence, Serum IL-6 could have a significant role in assessment of disease severity and Prognosis among COVID-19 patients.

Keywords: COVID-19, IL-6, Ferritin.

Introduction

Coronavirus disease 2019 (Covid-19) is a disease caused by the zoonotic novel Coronavirus called SARS-CoV-2[1]. Covid-19 quickly spread across the globe from a place of its origin in Hubei China and pandemic was declared by WHO on March 11, 2020[2]. Most patients with Covid-19 experience mild self-limiting disease. However, up to 20% of known cases of Covid-19 are complicated by severe pneumonia which might result in acute respiratory distress syndrome (ARDS) which causes acute hypoxemic respiratory failure [1, 3]. The overall infection fatality ratio is estimated to be below 1% [4]. It should be noted that fatal outcome may occur at any age including children and young adults [5]. Also, physiologic condition, pregnancy is associated with higher risk of severe disease [6]. However, the risk of death in Covid-19 is increasing with age and a presence of co-morbidities, especially cardiovascular diseases, diabetes mellitus and obesity [3]. According to data from the early China epidemic, the case-fatality ratio in the patients over 80 is up to 15% [7]. Elderly and highly co- morbid residents of long-term care facilities (LTCF) are at high risk of Covid-19 associated morbidity and mortality [8,10]. In one well-documented outbreak in LTCF in Washington, USA, more than half of the infected residents required hospitalization and more than one third died [10]. On the background of the ongoing pandemic, the spreading of Covid-19 in LTCF might significantly burden the local health care system and markedly contributes to mortality. Timely and effective intervention is essential to reduce morbidity and mortality during the Covid-19 outbreak in the LTCF. Such intervention consists of quick identification of cases, immediate introduction of infection control measures, initial triage, and daily monitoring of patients. An effective screening tool is essential to identify the patients at risk for severe ill- ness and death [11]. Such patients need close monitoring and early transfer to the hospital. Various biomarkers, especially inflammatory markers like C-reactive protein (CRP), ferritin, fibrinogen, D-dimer and Interleukin 6 (IL-6) are associated with Covid-19 progression [12,13]. According to known evidence, IL-6 is superior to CRP and other markers of inflammation in predicting respiratory failure in Covid-19[14]. IL-6 appears to be the most important driver of immune dysregulation and ARDS in Covid-19.

Material and methods

The cross-sectional analytical study was conducted in the Department of Pathology, Darbhanga Medical College and Hospital, Darbhanga, Bihar, India for 1 year, after taking the approval of the protocol review committee and institutional ethics committee. All COVID-19 positive patients, diagnosed upon admission by RT-PCR of oropharyngeal swabs with or without respiratory symptoms were included in the study group.

Inclusion and exclusion criteria

Patients younger than 18 years, critically ill patients like acute myocardial infarction patients during hospitalisation, Diabetes mellitus with acute complications, acute pancreatitis, chronic kidney disease were excluded from this study.

Sample size

Sample Size of 120 cases was estimated using n'master 2.0 sample size calculating software considering the IL-6 estimates as reported in a study by Chen X et al [15, 16].

Methodology

A total of 120 RT-PCR positive for COVID-19 virus were enrolled in the study and were segregated into three groups- Group A included asymptomatic patients and patients with mild symptoms with Respiratory Rate (RR) <24/m and SpO2 >94% in room air, Group B included symptomatic patients with mild to moderate pneumonia with RR: 24-30/m (or) SpO2: 90%-94% at Room Air, Group C included

symptomatic patient with severe pneumonia with ARDS RR >30/min (or) SpO2 <90% at Room Air (or) less than 94% with Oxygen, ARDS. This categorising of patients was based on the new clinical management protocol: COVID-19 guidelines by Government of India, Ministry of Health and family welfare, Directorate General of Health services. Version 5.2020 [17]. A detailed clinical history of Co morbidities like diabetes mellitus, cardiac disease, tuberculosis, bronchial asthma and arthritis if any were recorded.

Estimation

A 3 mL venous blood was collected from the patients with aseptic precautions, centrifuged at 3000 rpm for 5 minutes and the separated serum was used for Serum IL-6 estimation using Standard Enzyme Linked Immuno Sorbent Assay kits (Krishgen Bio Systems, India), as per kit instructions. Serum IL-6 levels were expressed in pg/mL.

Serum ferritin levels were estimated using automated clinical biochemistry analyser (Vitros ECi immunodiagnostic systems, Ortho clinical diagnostics, United Kingdom). Serum Ferritin levels expressed in ng/mL.

Statistical analysis

Continuous data was presented with mean, standard deviation and confidence interval. The Data was checked for normality and Mann-Whitney U test, and Kruskal Wallis Test was used to test significance of difference between the groups mean. A p-value <0.05 was considered as statistically significant. IBM SPSS 21.0 version software was used for data analysis are presented in tables.

Table 1: Gender and age distribution of patients

Gender	**Number**	**%**
Male	80	66.67
Female	40	33.33
Age in years		
20-40	25	20.83
40-60	69	57.5
Above 60	26	21.67

The number of male patients was 80 and female patients were 40 (Table 1). 120 COVID-19 infected patients confirmed by RT-PCR. A total of 25 patients were aged between 20-40 years, 69 patients between 40-60 years age group and 26 patients were in 60-80 years of age group.

Table 2: Correlation analysis of Serum IL-6 Levels and Serum Ferritin levels in Group A, Group B and Group C patients with Mild, Moderate and Severe disease of COVID-19 infection; Mann-Whitney U test and Kruskal Wallis pairwise comparison.

Parameters		**N**	**Mean ±Std. Deviation**	**Kruskal Wallis p-value**	**Mann-Whitney (pairwise) p-value**
IL-6	Group A	55	11.36±2.77	<0.001***	<0.001***
	Group B	30	42.02±10.69		
	Group C	35	123.66±36.98		
Ferritin	Group A	55	372.63±369.55	0.42	0.44
	Group B	30	311.54±289.69		
	Group C	35	436.87±381.63		

Serum IL-6 was an indicator of COVID-19 disease severity (p<0.001 ***highly significant) compared to Serum Ferritin.

Group A had 55 patients among which 7 patients were asymptomatic and 48 had mild symptoms with RR <24/m and SpO2 >94% in room air, the number of patients with co-morbidities like type 2 diabetes mellitus were 15, with hypertension and cardiac disorders were 10 and with respiratory disorders were 5 in Group A, Group B there were 30 patients among which 9 had mild symptoms and 21 patients had moderate symptoms with RR: 24-30/m (or) SpO2: 90%-94% at room air. The mean levels with standard deviation of Serum IL-6 and serum ferritin in Group A, Group B and Group C patients, respectively has depicted in. (Table 2). On pairwise comparison by Mann-Whitney U test among the groups it shows that the mean IL-6 levels are significantly different in all the three groups. Kruskal Wallis pairwise comparison shows IL-6 levels to be significantly increased in Group C (35) patients with severe disease compared to Group A and Group B patients with mild and moderate disease respectively (p-value <0.001). Serum Ferritin levels did not show any statistically significant difference among Group A, Group B and Group C, however ferritin levels were markedly increased in Group C patients with severe disease (p-value=0.44) (Table 2).

Group A patients were managed with Face mask/Nasal Prongs, Group B patients were managed with high flow nasal cannula/ Bi level Positive Airway Pressure (BIPAP), Group C patients required mechanical ventilation/high flow nasal cannula.

Discussion

In the present study, 120 RT-PCR confirmed COVID-19 infection cases, the patients had presented with mild symptoms to severe pneumonia. The patients with severe symptoms required ICU Admission. Around admitted to the ICU under mechanical ventilation died due progression of the COVID-19 infection to severe ARDS.

In this cross-sectional analytical study, the levels of IL-6 were found to be significantly elevated in COVID-19 infected patients presented with severe symptoms, the mean levels of IL-6 were consistently increasing in mild to moderate and from moderate to severely infected COVID-19 patients. The findings in this study were in par with the retrospective study of dynamic changes in IL-6 levels in COVID-19 patients observed by Liu Z et al., on 738 COVID-19 Patients admitted in the hospital at Wuhan, China. The author observed increased levels of IL-6 that significantly correlated with disease severity and hospital mortality due to COVID-19 infection [18]. As far as we have understood the pathogenesis of COVID-19 infection. The corona virus binds with the Angiotensin Converting Enzyme 2 (ACE2) receptors on the surfactant producing Type II Alveolar cells, ciliated and goblet cells in the airways [19-21]. The corona virus effectively inhibits type 1 Interferon (IFN) signaling that will suppress the antiviral programs and subsequently activate innate and adaptive immune response that induces the release of Proinflammatory cytokines through NF kB pathway. The proinflammatory cytokines include IL-1, IL-6, and TNF α later increases the vascular permeability, causing influx of large amounts of fluid and blood cells into the alveoli causing ARDS [22,23]. In this study, any significant clinical correlation with ferritin levels among COVID-19 patients could not found. However, it was found that the serum ferritin levels were quite high in severe cases as compared with mild to moderately symptomatic COVID-19 infection. The present study findings were in par with observations made by Zhon F et al., where Ferritin levels of >400 correlated significantly with severe infection and mortality due to COVID-19. The increased ferritin levels in patients with severe COVID-19 infection may indicate a secondary bacterial infection, resulting in increased synthesis and release of intracellular ferritin especially from the

reticuloendothelial system during viral and bacterial infections [24]. Ferritin is a protein concerned with iron storage and homeostasis, serum ferritin is also an acute phase protein found to be elevated during infections, as a part of host defense mechanisms, hyper ferritinaemia in infections is protective as it limits the production of free radicals and mediates immunomodulation [25]. Previous studies show hyperferritinemia in dengue virus infection to indicate a highly active disease with immune activation and coagulation disturbances [26,27].

Similarly, in COVID-19 the release of excessive of ferritin from cells is observed to be due to cytokine stimulus especially IL-6, as an account of viraemia there is significant activation of macrophages. This hyperferritinemia is seen in severe infections of COVID-19 and its levels correlates with high mortality [28,29]. This study could not find any significant correlation between serum IL-6 levels with Ferritin levels across disease severity taken together. However, there was a proportionate increase in the levels of IL-6 with regards to the severity in manifestations of COVID-19 infected patients in the present study. Hence, determining the IL-6 levels in COVID-19 patients can be a potentially useful in clinically correlating with the severity of the infection and timely management.

Conclusion

Serum IL-6 levels independently showed a good correlation with disease severity among COVID-19 patients, and serum ferritin levels was elevated only in severely symptomatic individuals with COVID-19 infections. Hence, Serum IL-6 could have a significant role in assessment of disease severity and Prognosis among COVID-19 patients.

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