Serum IL-6 as a Vital Predictor of COVID-19 Severity in Iraqi Patients

Haifa Hmood Kareem*, Batool Hassan Al-Ghurabi

Department of Basic Science, College of Dentistry, University of Baghdad, Baghdad, Iraq

ABSTRACT

Background: During the great attack, Iraq first reported a confirmed SARS-CoV-2 infection in Najaf on February, 2020. COVID-19 stimulates the production of pro-inflammatory cytokines such as IL-6 which is a mediator of lung inflammation.

Aim of study: The aim of this study was to estimate IL-6 levels and examine it's associated with COVID-19 severity.

Materials and Methods: 50 patients with COVID-19 and 35 healthy controls were participated in this case control study, their age range from 18 to 77 years. Oral hygiene index was used to determine oral health status. Serum obtained from patients and controls was analysed using ELISA to assess levels of IL-6.

Results: The present study found that there is significant elevation (P<0.05) in the median serum level of IL-6 among patients with COVID-19 as compared to healthy controls. As well, there is a significant difference (p<0.05) between severe group and each of moderate and mild groups, while there is no significant difference between moderate and mild group. Concerning oral hygiene, the level of serum IL-6 is significantly increased (p<0.05) in patients with poor oral hygiene than those patients with good oral hygiene.

Conclusion: Elevated IL-6 levels are associated with worse infection with SARS-CoV-2 and are an important predictor for COVID-19 patients in severe stage.

Key words: SARS-CoV-2, COVID-19, cytokines, IL-6

INTRODUCTION

Coronavirus disease 2019 (COVID-19) is an infectious disease caused by SARS-CoV-2, first identified in December 2019 in China, and has since spread all over the world, resulting in the on-going 2019-2020 coronavirus pandemic [1]. COVID-19 affects hundreds of thousands of individuals, having life-threatening outcomes, not only in medically compromised persons but also in perfectly healthy young individuals with immune competent system. Apparently, this specific coronavirus must possess special abilities to spread and compromise the immune mechanisms in humans [2]. Illness ranges in severity from asymptomatic or mild to severe; a significant proportion of patients with clinically evident infection develop severe disease, which may be complicated by acute respiratory distress syndrome and shock. Mortality rate among diagnosed cases (case fatality rate) is generally about 2% to 3% but varies by country; true overall mortality rate is uncertain, as the total number of cases (including undiagnosed persons with milder illness) is unknown [3].

Blanco-Mello and colleagues described a distinctive and unsuitable inflammatory response related to SARS-CoV-2 infection. The authors revealed that an inappropriate and weak immune response appears more frequently in patients with comorbidities. Thus, this could favour virus replication and enhance complications related to severe cases of the disease [4,5]. In the short time since the emergence of COVID-19, numerous studies have described abnormal levels of the cytokines and chemokines in the patients [6,7]. The key point in SARS-CoV-2 infection could be the depletion of antiviral defences related to innate immune response as well as an elevated production of inflammatory cytokines [4]. Recently demonstrated that COVID-19 stimulates the production of pro-inflammatory cytokines such as Interleukin (IL)-1β and IL-6 *via* the Toll like Receptors (TLR) that causes the release of active IL-6 that is a mediator of lung inflammation. Increased concentrations of IL-6 is associated with disease severity and course [8,9] and also associated with death in patients with severe COVID-19 infection [10,11]. Therefore, the aim of this study was to estimate IL-6 levels and examine it's associated with COVID-19 severity.

MATERIALS AND METHODS

Patients group: In this study, 50 COVID-19 patients (29 males and 21 females) were included, ranging in age from 18 to 77 years. They were admitted to Baghdad teaching

hospital/medical city from November 2020 to January 2021. All patients were diagnosed with SARS-CoV-2 infection, according to the World Health Organization criteria [12]. Real-Time Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) assay was used to identify SARS-CoV-2 infection. The clinical classification of patients was categorized by disease severity into mild, moderate and severe, according to sign and symptoms by clinical management guidelines outlined in the diagnosis and treatment protocol for COVID-19. Inclusion criteria; the patients enrolled in this study and considered eligible must have met the following criteria; COVID-19 infection signs and symptoms (fever, generalized malaise, cough and difficulty breathing) as well as COVID-19 RT-PCR. This study excluded children and pregnant women, patients with chronic viral infections and systemic disorders, allergic rhinitis and chronic sinusitis, and patients who were unable to give informed consent.

Controls group: consisted of 35 individuals (16 males and 19 females), their ages and sexes were matched to patients their ages ranged between (18-73) years.

Oral hygiene status was determined by the simplified oral hygiene index "Oral Hygiene Index=Debris Index +Calculus Index" [13].

Sample collection: Four millilitre of venous blood was collected from all participants. Blood was transferred to sterile plain tube, and serum was separated by centrifugation at 3000 rpm for 10 minutes, then divided into small aliquots and kept at -20ºC until used for analysis.

InterLeukin-6: Serum IL-6 was estimated by ELISA and done according to the instructions on the kit's booklet.

Statistical analysis: The data was non-parametric and described by median, as evidenced by histograms and the Smemirnove-Kolmogorove test, and non-parametric tests of significance were recommended. Statistical significance was defined as a P value of less than 0.05.

RESULTS

The present study found that there is significant elevation (P<0.024) in the median serum level of IL-6 among patients with COVID-19 (42.68 pg/ml) as compared to healthy controls (36.71 pg/ml), (Table 1). As well, results in Table 2 showed a significant difference (p<0.05) in median serum level of IL-6 among three groups of patients, the median level of severe cases was (84 pg/ml), for moderate (41 pg/ml) and mild cases was (44 pg/ml). On the other hand, there is a significant difference (p<0.05) between severe group and each of moderate and mild groups, while there is no significant difference between moderate and mild group.

Table 1: Comparison of sickled RBC in anti-sickling analysis of two methods with pre-treatment (n=49).

Serum IL-6	Study groups		P-value
	COVID-19 patients	**Healthy control**	
	N=50	N=35	
Min	13.57	22.53	
Max	561.25	545.06	0.024[*]
Median	42.68	36.71	
Mean Rank	48.05	36.87	
[*]:Significant, No: Number			

Table 2: Serum levels of IL-6 (pg/ml) in patients group according to disease severity.

Serum IL-6	Patients group			
	Severe	**Moderate**	**Mild**	**P-value**
	N=10	**N=29**	**N=11**	
Min	44	13	26	
Max	233	561	121	
Median	84	41	44	0.019[*]
Mean Rank	28.05	17.22	19.82	
Severe group *vs.* moderate group				0.010[*]
Severe group *vs.* mild group				0.016[*]
Moderate group *vs.* mild group				0.833 NS
NS: Non–Significant,				

Concerning oral hygiene, the level of serum IL-6 is significantly increased (p<0.05) in patients with poor oral hygiene was (77 pg/ml) than those patients with good oral hygiene (41 pg/ml), as demonstrated in Table 3.

Table 3: Serum levels of IL-6 (pg/ml) in patients group according to oral hygiene.

Serum IL-6	Good	Poor
	N=18	N=32
Min	26	26
Max	161	356
Median	41	77
Mean Rank	19.72	29.42
P-value	0.026*	

DISCUSSION

Not surprisingly, research into SARS-CoV-2 infection is currently the major priority for science communities worldwide. So this encouraged us to study the role of IL-6 in COVID-19 patients with different stages of disease. Although this was the main aim of the present study but nevertheless the impact of oral health status in COVID-19 patients compared to healthy controls was also investigated.

The present results found that the levels of IL-6 are significantly higher in COVID-19 patients than those in healthy control. As well, there is significant increase in level of IL-6 among patients with severe cases than that in moderate and mild cases. The present observations were in line with previous findings reported that the level of IL-6 was increase in patients with SARS-CoV-2 infection [14-16]. Showed that the highest level of IL-6 was found in newly diagnosed COVID-19 patients comparing with healthy control [17]. In addition, Cazzolla reported a significant directly proportional correlation between IL-6 levels and the presence of self-reported olfactory dysfunction [18]. However, the elevated cytokine levels may also be responsible for the lethal complications of COVID-19, patients with COVID-19, SARS or MERS presented distinct cytokine profiles. Patients with COVID-19 presented elevated T helper 2 cytokines (IL-4) in addition to T helper 1 cytokines compared to those in patients with SARS or MERS [19].

Moreover, a study of 452 patients infected with SARS-CoV-2 reported that the elevation of IL-6 levels was more marked with more severe symptoms [20]. As well Sabaka revealed that patients with severe COVID-19 had a high mean level of IL-6 compared to patients in mild disease, this was in agreement with the results of the current study. Serum IL-6 concentrations were associated with a greater likelihood of progression to critical illness status, indicating the need for more active interventions to prevent further deterioration to a life-threatening condition [21]. On the other hand, pointed out to that elevated IL [23]. Mechanistically, IL-6 is essential for the generation of T helper 17 (Th17) cells in the dendritic cell-T cell interaction [24]. The excessive IL-6 may explain the overly activated Th17 cells observed in COVID-19 patients [25]. Animal studies of SARS-CoV have demonstrated that inhibiting nuclear factor kappa-B (NF-κB), a key transcription factor of IL-6, or infecting animals with SARS-CoV lacking the CoV E protein, a strong stimulus to NF-κB signalling, increased animal survival, with reduced IL-6 levels [26].

The present study also indicated that the serum IL-6 level was significantly higher in patients with COVID-19 poor oral hygiene than in patients with good oral health this could explain in part due to that pro-inflammatory cytokines levels were correlated with oral health status and the presence of oral microorganisms. Unfortunately, no previous studies with such comparison were found. In this context, periodontitis has been linked to increased levels of pro-inflammatory cytokines, including IL-6, which is a recognized mediator in the periodontal destruction process [27]. The ability of nonsurgical periodontal treatment to lower cytokine levels has been highlighted in the dental literature [28,29]. Thus, lower IL-6 levels and inflammation resulting from periodontal treatment can potentially protect COVID-19 patients against life threatening respiratory complications. In fact, previous studies have linked periodontitis to respiratory conditions, with increased risk for complications and higher mortality rate in hospitalized patients [30]. The impact of a connection between periodontal treatment, IL-6 levels, respiratory conditions and COVID-19 can be powerful considering the high prevalence of periodontal disease in adults, the high transmission rate of SARS-CoV-2, the limited access to periodontal treatment during the pandemic and the shortage of health care resources related to hospitalized COVID-19 patients who require mechanical ventilation [31].

CONCLUSION

In conclusion elevated IL-6 levels are associated with worse infection with SARS-CoV-2 and are an important predictor for COVID-19 patients in severe stage.

COMPETING INTERESTS

The authors declare that they have no competing interests.

REFERENCES

1. Hui DS, Azhar EI, Madani TA, et al. The continuing 2019-nCoV epidemic threat of novel coronaviruses to global health-The latest 2019 novel coronavirus outbreak in Wuhan, China. Int J Infect Dis 2020; 91:264-266.

2. Prompetchara E, Ketloy C, Palaga T. Immune responses in COVID-19 and potential vaccines: Lessons learned from SARS and MERS epidemic. Asian Pac J Allergy Immunol 2020; 38:1-9.

3. Oke J, Heneghan C. Centre for Evidence-Based Medicine. Global COVID-19 Case Fatality Rates. CEBM 2020.

4. Blanco-Melo D, Nilsson-Payant BE, Liu WC, et al. Imbalanced host response to SARS-CoV-2 drives development of COVID-19. Cell. 2020; 181:1036-1045.

5. Huang C, Wang Y, Li X, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. The lancet 2020; 395:497-506.

6. Adham ZS, Al-Ghurabi BH. Serum Level of Cathelicidin in COVID-19 Patients in Relation with Oral Heath. J Res Med Dent Sci 2021; 9:85.

7. Chen C, Zhang X, Ju Z, et al. Research progress on the mechanism of cytokine storm induced by new coronavirus pneumonia and related immunotherapy [JOL]. Chin J Burns 2020; 36:E005.

8. Conti P, Ronconi G, Caraffa AL, et al. Induction of pro-inflammatory cytokines (IL-1 and IL-6) and lung inflammation by Coronavirus-19 (COVI-19 or SARS-CoV-2): anti-inflammatory strategies. J Biol Regul Homeost Agents 2020; 34:327-331.

9. Zumla A, Hui DS, Azhar EI, et al. Reducing mortality from 2019-nCoV: host-directed therapies should be an option. The Lancet 2020; 395:35-36.

10. McGonagle D, Sharif K O Regan A, et al. The role of cytokines including interleukin-6 in COVID-19 induced pneumonia and macrophage activation syndrome-like disease. Autoimmun Rev 2020; 19:102537.

11. Zhang ZL, Hou YL, Li DT, et al. Laboratory findings of COVID-19: a systematic review and meta-analysis. Scand J Clin Lab Inv 2020; 80:441-447.

12. World Health Organization. Coronavirus disease 2019 (COVID-19). 2020.

13. Greene JG, Vermillion JR. The simplified oral hygiene indexJ Am Dent Assoc 1964; 68:7-13.

14. Del Valle DM, Kim-Schulze S, Huang HH, et al. An inflammatory cytokine signature predicts COVID-19 severity and survival. Nat Med 2020; 26:1636-1643.

15. Herold T, Jurinovic V, Arnreich C, et al. Elevated levels of IL-6 and CRP predict the need for mechanical ventilation in COVID-19. J. Allergy Clin Immunol Pract 2020; 146:128-136.

16. Zhang J, Hao Y, Ou W, et al. Serum interleukin-6 is an indicator for severity in 901 patients with SARS-CoV-2 infection: a cohort study. J Transl Med 2020; 18:1-8.

17. Kathim MJ, Taha TA, Hussain SS, et al. Il-6, il-0, ifn gamma and crp in newly diagnosed COVID-19 patients. Medico-Legal Update. 2021; 1418-1422.

18. Cazzolla AP, Lovero R, Lo Muzio L, et al. Taste and smell disorders in COVID-19 patients: role of interleukin-6. ACS Chemical Neurosci 2020; 11:2774-2781.

19. Costela-Ruiz VJ, Illescas-Montes R, Puerta-Puerta JM, et al. SARS-CoV-2 infection: The role of cytokines in COVID-19 disease. Cytokine and growth factor reviews. 2020; 54:62-75.

20. Qin C, Ziwei MP, Tao SY, et al. Dysregulation of immune response in patients with COVID-19 in Wuhan, China; Oxford Academic. Clin Infect Dis 2020.

21. Sabaka P, Koscalova A, Straka I, et al. Role of interleukin 6 as a predictive factor for a severe course of Covid-19: retrospective data analysis of patients from a long-term care facility during Covid-19 outbreak. BMC infect dis 2021; 21:1-8.

22. 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.

23. Zhou J, He W, Liang J, et al. Association of interleukin-6 level with morbidity and mortality in patients with coronavirus disease 2019 (COVID-19). Jpn J Infect Dis 2020.

24. Tanaka T, Narazaki M, Kishimoto T. Immunotherapeutic implications of IL-6 blockade for cytokine storm. Immunotherapy 2016; 8:959-970.

25. Xu Z, Shi L, Wang Y, et al. Pathological findings of COVID-19 associated with acute respiratory distress syndrome. The Lancet res med 2020; 8:420-422.

26. DeDiego ML, Nieto-Torres JL, Regla-Nava JA, et al. Inhibition of NF-κB-mediated inflammation in severe acute respiratory syndrome coronavirus-infected mice increases survival. J Virol. 2014; 88:913-924.

27. Mahmood HK, Al-Ghurabi BH. Association between anti-CMV IgG and salivary levels of IL-6 and TNF-α in chronic periodontitis. JBCD 2020; 32:5-11.

28. Reis C, Da Costa AV, Guimarães JT, et al. Clinical improvement following therapy for periodontitis:

Association with a decrease in IL-1 and IL-6. Exp Ther Med 2014; 8:323-327.

29. Zhou SY, Duan XQ, Hu R, et al. Effect of non-surgical periodontal therapy on serum levels of TNF-α, IL-6 and C-reactive protein in periodontitis subjects with stable coronary heart disease. Chin J Dent Res 2013; 16:145-151.

30. Hobbins S, Chapple IL, Sapey E, et al. Is periodontitis a comorbidity of COPD or can associations be explained by shared risk factors/behaviors? Int J Chron Obstructive Pulmon Dis 2017; 12:1339.

31. White DB, Lo B. A framework for rationing ventilators and critical care beds during the COVID-19 pandemic. Jama 2020 ;323:1773-1774.