Academicia Globe:
Inderscience Research

ISSN: 2776-1010 Volume 4, Issue 2, Feb., 2023

"SALIVARY INTERLEUKIN-6 AS A BIOMARKER OF COVID-19 SEVERITY"

Bahaa Noor Madhloom
MSc., Oral Medicine, Faculty of Medicine, Jabir Ibn Hayyan Medical University, Al Najaf, Iraq ,
E-mail: bahaa.n.madhloom@jmu.edu.iq,
bahaanoorm@gmail.com.Orcid: 0000-0002-5785-1901

Abstract:

Background: The "cytokine storm" induced by SARS-CoV-2 infection is known to cause significant lung damage and even death in extreme situations. Thus, an early clinical prognosis is sought to reduce the overproduction of inflammatory cytokines. The most significant proinflammatory cytokines that are increased in COVID-19 are interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α). Considering the recent interest in and benefits of using saliva as a clinical specimen, i.e., the convenience and painlessness of collection, this does not necessarily require trained personnel and could potentially permit self-sampling.

Objective: The purpose of this study is to look at the possible changes in IL-6 and TNF-α found in the saliva of COVID-19 patients and see if they can be used as a non-invasive biomarker for the severity of the condition.

Method: This case-control study contains 90 subjects; a total of 60 COVID-19 individuals with varying severity levels met the inclusion criteria. A control group consisting of 30 individuals of the same age and gender was established. Blood and saliva samples were taken in pairs from both the control and study groups, in accordance with routine sampling procedures. The current study looks into the detection of IL-6 and TNF-α in saliva using ELISA. Blood samples from the same participants were evaluated concurrently to compare and validate the expression of inflammatory markers.

Results: IL-6 protein and TNF- levels in patients' blood and saliva were significantly higher than in healthy controls ($P<0.001$). Surprisingly, high saliva IL-6 and TNF-α levels were linked to COVID-19 severity ($P<0.001$).

Conclusion: The findings show that high salivary IL-6 and TNF-α levels are linked to COVID-19 infectivity and disease severity. This result implies that saliva may be a viable alternative to blood for monitoring inflammation in COVID-19 patients.

Keywords: Coronavirus disease 2019, Interleukin-6, TNF-α, Saliva and Severe acute respiratory syndrome-coronavirus-2

Academicia Globe:
Inderscience Research

ISSN: 2776-1010 Volume 4, Issue 2, Feb., 2023

Introduction:

COVID-19 pneumonia may proceed to acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) due to excessive inflammatory responses (1-3). The present care of COVID-19 is supportive; consequently, it is recommended that all patients with severe COVID-19 be tested for hyper-inflammation or "cytokine storm" to identify individuals who would benefit from targeted immunosuppressive medications or immunomodulatory therapy to prevent ALI/ARDS (4).

TNF alpha (TNF-), interleukin-6 (IL-6), neutrophil chemoattractant such as interleukin-8 (IL-8) and monocyte chemoattractant protein-1 (MCP-1, CCL2) are all produced when T helper 17 (Th-17) cells are dysregulated. Recruited neutrophils then produce reactive oxygen species, resulting in ALI and protein-rich inflammatory lung infiltration, which are the defining characteristics of ARDS (5, 6).

Multiple studies have also linked the elevated levels of IL-6 and the Th17 response in the upper and lower respiratory tracts of COVID-19 patients to the severity of the disease. Additionally, we and others have demonstrated that the amounts of many plasma indicators can be effectively represented in saliva (7, 8).

As a result, we hypothesized that salivary IL-6 and TNF-α levels could mimic plasma levels and thus serve as noninvasive biomarkers for disease severity.

A growing body of clinical evidence acknowledges that an increased immune response known as a "cytokine storm" is connected with the severity of COVID-19 and is regarded as an indisputable cause of mortality (9, 10). It has long been believed that cytokines play a vital role in the immunopathology of viral infections. The first line of defense versus viral infection is a prompt and very well innate immune response. However, inappropriate and dysregulated immune responses result in tissue damage (11). Several studies have demonstrated the importance of inflammatory markers in COVID-19 severity and progression. Plasma levels of IL-2, IL-6, and TNF-α were higher in severe COVID-19 cases than in mild cases, indicating that measuring inflammatory cytokine release is critical for understanding the course and severity of COVID-19 (12). An early COVID-19 study also discovered that activated mast cells in the respiratory tract submucosa produced proinflammatory cytokines such as IL-6, TNF-α, and even IL-10, which exacerbated the inflammatory state and contributed to pathogenesis (13-15).

The shown method of using saliva as a sample to find and track inflammation could help with early detection and systematic monitoring of inflammatory markers, which could lead to a simple and quick diagnosis that could help doctors take care of patients more efficiently.

Academicia Globe:
Inderscience Research

ISSN: 2776-1010 Volume 4, Issue 2, Feb., 2023

Method:

Design of the study and sample collection:

The study included sixty adults aged 19 to 71 with PCR-confirmed SARS-CoV-2 infection who were referred to ALHAKIM Hospital in Najaf in 2022. Thirty individuals exhibited mild to moderate symptoms, while thirty patients exhibited severe disease. The severity status of COVID-19 was described as "COVID-19 pneumonia necessitating high-flow oxygen delivery" (16). The control group comprised thirty healthy individuals. Both saliva and blood samples were taken from 90 participants. For IL-6 and TNF-α concentrations, blood and saliva samples were tested using an ELISA (enzyme-linked immunosorbent assay). Also assessed in COVID-19 individuals were serum D-dimer and CRP.

Biostatistical analysis:

IBM SPSS 26.0 was used to conduct the statistical analysis. Frequency and proportion are used to express categorical variables. The mean and standard deviation are used to depict continuous variables.

Results:

Sixty patients with PCR-confirmed SARS-CoV-2 infection, 30 with acutely severe COVID-19 pneumonia, and high serum indicators of COVID-19 severity, including D-dimer and CRP the clinical parameters of the patients are reported in Table 1.

Table 1. Clinical parameters of COVID-19 patients.

variables	Control group	Study group		P-value
		Mild/ moderate (n-30)	Sever (n= 30)	
Age (years, mean, range)	31.26+-2.6	36.3+-1.6	60.5+-6.2	
Salivary flow rate	0.43+-0.08	0.41+-0.08	0.35+-0.07	
Serum inflammatory markers				
D-dimer (0–0.5 μ mL–1)	----	0.91+_0.3	5.35+-2.2	<0.001
CRP (1.0–3.0 mg L–1)	-----	42.7+-24.9	37.8+-30.9	<0.001
Cytokines values				
Plasma IL-6, pg mL–1	47.08+_3.09	110.45+_23.9	226.57+_61.9	<0.001
Saliva IL-6, pg mL–1	16.16+_3.14	33.6+_11.5	160.08+_41.5	<0.001
plasma TNFα, pg.mL-1	13.67+_0.55	179.8+_59.1	371.13+_113.9	<0.001
Saliva TNFα, pg.mL-1	3.35+_0.58	54.03+_4.58	124.02+_32.01	<0.001

Academicia Globe:
Inderscience Research

ISSN: 2776-1010 Volume 4, Issue 2, Feb., 2023

IL-6 levels in patients' blood and saliva were substantially higher than in healthy individuals (P<0.001). Similarly, TNF-α concentrations in patients' blood and saliva were significantly higher than in healthy individuals (P<0.001).

Plasma protein levels (D-dimer and CRP) were substantially higher in severe COVID-19 individuals than in mild or moderate COVID-19 cases (P<0.001). Also Positive correlation was observed between salivary IL-6 levels and plasma protein (D-dimer and CRP) (r^2=0.674, r^2=0.159. fig1 & fig2). Furthermore a positve correlation between salivary and serum IL-6 and TNF-α respectively (r^2=0.446, r^2=0.332) (fig3 & fig4).

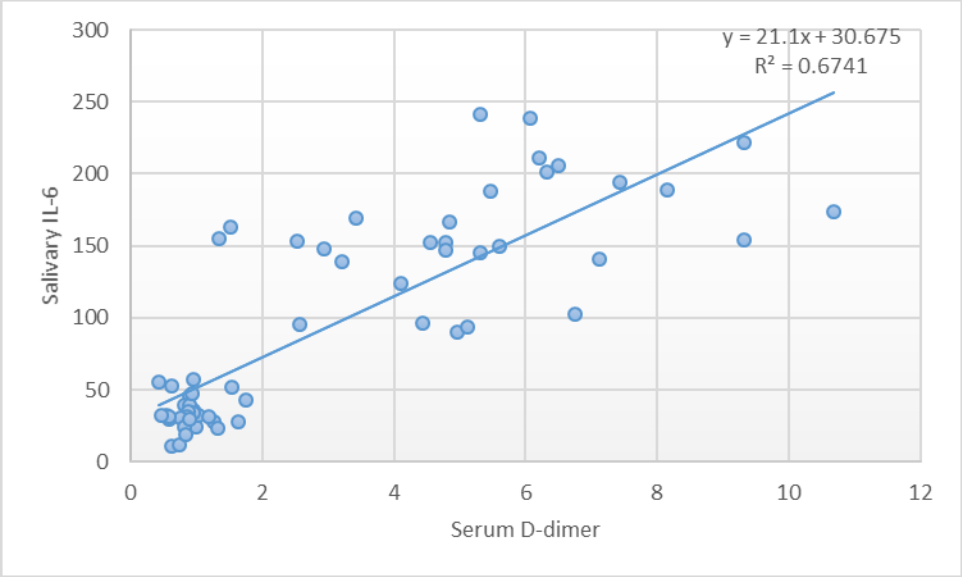

Fig1: correlation between Salivary IL-6 and Serum D-dimer for patients

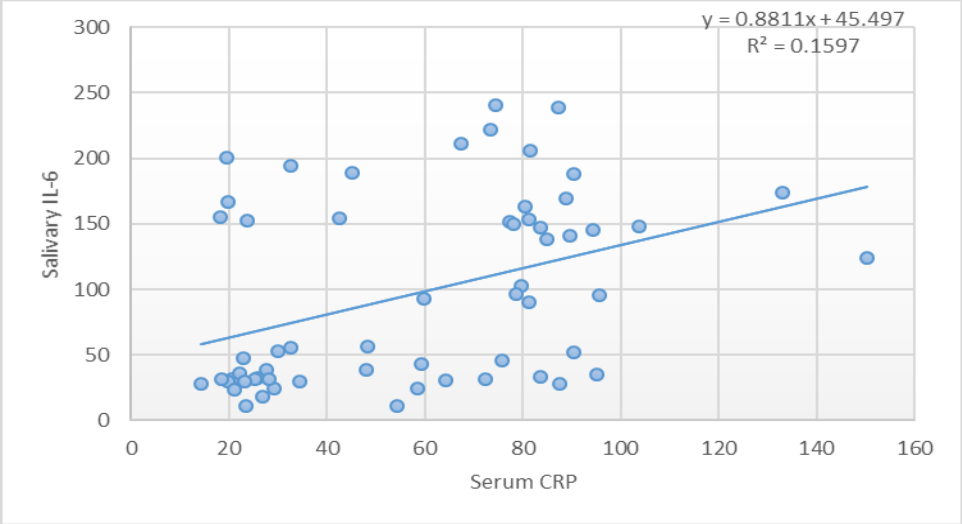

Fig2: correlation between Salivary IL-6 and Serum CRP for patients

Academicia Globe:
Inderscience Research

ISSN: 2776-1010 Volume 4, Issue 2, Feb., 2023

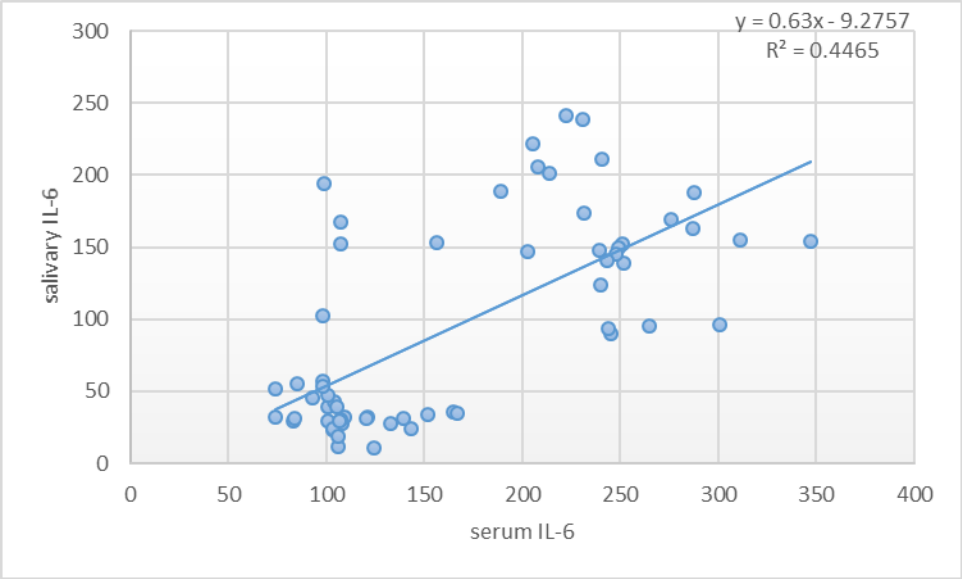

Fig3: correlation between Salivary IL-6 and Serum IL-6 for patients

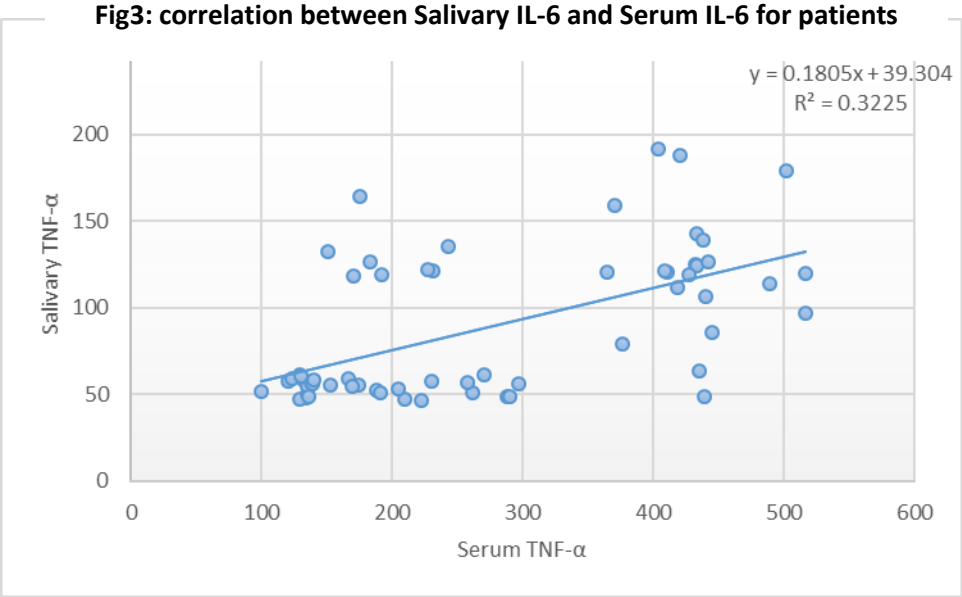

Fig4: correlation between Salivary TNF-α and Serum TNF-α for patients

Discussion:

In the present investigation, we discovered that the amount of IL-6 in the saliva of COVID-19 patients mirrored its level in the blood. Higher IL-6 levels in COVID-19 patients' saliva were associated with disease severity and poorer clinical outcomes, regardless of IL-6 or TNF-α level.

Saliva has been proposed as a potential non-invasive biological sample for the diagnosis of SARS-CoV-2 (17). Different respiratory viruses thrive in salivary fluid.

ISSN: 2776-1010 Volume 4, Issue 2, Feb., 2023

Additionally, virus particles can be transferred from the upper and lower respiratory tracts to the saliva (18). In addition to virus particles, salivary fluid contained significant prognostic inflammatory markers such as CRP and TNF-α (19). Salivary glands are surrounded by numerous blood vessels that promote the exchange of blood and salivary fluid (20). Blood proteins have been demonstrated to access saliva intracellularly via passive diffusion or active transport and paracellularly by ultrafiltration at the tight junctions of salivary gland cells. Saliva is hypotonic relative to plasma (21). Therefore, the high amount of IL-6 reported in the saliva of severe COVID-19 cases may be directly caused by active SARS-CoV-2 infection within the oral cavity and salivary gland, in addition to the protein that may have diffused from the bloodstream.

Notably, we found that IL-6 saliva level represents a potential biomarker of COVID-19 severity and inferior survival outcomes, even after considering for other risk factors, such as patient demographics and COVID-19 severity markers. TNF-α is a known inflammatory marker and organ injury, and people with COVID-19 have high TNF-α levels in their blood (22).

Conclusion:

Saliva is recognized as a mirror that reveals an individual's physical condition and as a possible specimen for the diagnosis and prognosis of many disorders. The current study investigated the viability of saliva as a specimen for detecting IL-6 and TNF- in COVID-19 patients using the ELISA method. The results are favorable and justify the use of saliva in this setting as opposed to blood.

By focusing on the identification of inflammatory markers, this investigation also motivates additional scientists and researchers to pursue studies relevant to salivary diagnostics in diverse medical states.

Refrences:

1. Salto-Tellez M, Tan E, Lim B. ARDS in SARS: cytokine mediators and treatment implications. Cytokine. 2005; 29(2):92–4. https://doi.org/10.1016/j.cyto.2004.09.002 PMID: 15598444.

2. Ksiazek TG, Erdman D, Goldsmith CS, Zaki SR, Peret T, Emery S, et al. A Novel Coronavirus Associated with Severe Acute Respiratory Syndrome. N Engl J Med. 2003; 348(20):1953–66. https://doi.org/10.1056/NEJMoa030781 PMID: 12690092

3. Yang X, Yu Y, Xu J, Shu H, Xia Ja, Liu H, et al. Clinical course and outcomes of critically ill patients with SARS-CoV-2 pneumonia in Wuhan, China: a single-centered, retrospective, observational study. The Lancet Respiratory Medicine. 2020; 8(5):475–81. https://doi.org/10.1016/S2213-2600(20)30079-5 PMID: 32105632.

4. Mehta P, McAuley DF, Brown M, Sanchez E, Tattersall RS, Manson JJ. COVID-19: consider cytokine storm syndromes and immunosuppression. The Lancet. 2020; 395(10229):1033–4. https://doi.org/1016/S0140-6736(20)30628-0 PMID: 32192578

ISSN: 2776-1010 Volume 4, Issue 2, Feb., 2023

5. Veldhoen M. Interleukin 17 is a chief orchestrator of immunity. Nat Immunol. 2017; 18(6):612–21. https://doi.org/10.1038/ni.3742 PMID: 28518156
6. Pacha O, Sallman MA, Evans SE. COVID-19: a case for inhibiting IL-17? Nature Reviews Immunology. 2020; 20(6):345–6. https://doi.org/10.1038/s41577-020-0328-z PMID: 32358580.
7. Saheb Sharif-Askari F, Saheb Sharif Askari N, Goel S, Mahboub B, Ansari AW, Temsah M-H, et al. Upregulation of IL-19 cytokine during severe asthma: a potential saliva biomarker for asthma severity. ERJ Open Research. 2021:00984–2020.
8. Galhardo LF, Ruivo GF, de Oliveira LD, Parize G, Santos SSFD, Pallos D, et al. Inflammatory markers in saliva for diagnosis of sepsis of hospitalizes patients. Eur J Clin Invest. 2020; 50(5):e13219. https://doi.org/10.1111/eci.13219 PMID: 32129475.
9.Hu, B., S. Huang, and L. Yin. 2021. The cytokine storm and COVID-19. Journal of Medical Virology 93 (1): 250–256. https://doi.org/10.1002/jmv.26232.
10. Bhaskar, S., A. Sinha, M. Banach, S. Mittoo, R. Weissert, J.S. Kass, S. Rajagopal, A.R. Pai, and S. Kutty. 2020. Cytokine storm in COVID-19-immunopathological mechanisms, clinical considerations, and therapeutic approaches: The REPROGRAM consortium position paper. Frontiers in Immunology 11: 1648. https://doi.org/10.3389/fmmu.2020.01648.
11. Ye, Q., B. Wang, and J. Mao. 2020. The pathogenesis and treatment of the 'cytokine storm' in COVID-19. The Journal of Infection 80 (6): 607–613. https://doi.org/10.1016/j.jinf.2020.03.037.
12. Xiong, Y., Y. Liu, L. Cao, D. Wang, M. Guo, A. Jiang, D. Guo, W. Hu, J. Yang, Z. Tang, H. Wu, Y. Lin, M. Zhang, Q. Zhang, M. Shi, Y. Liu, Y. Zhou, K. Lan, and Y. Chen. 2020. Transcriptomic characteristics of Broncho-alveolar lavage fuid and peripheral blood mononuclear cells in COVID-19 patients. Emerging Microbes & Infections 9 (1): 761–770. https://doi.org/10.1080/22221751.2020.1747363.
13. Conti, P., G. Ronconi, A. Carafa, C. Gallenga, R. Ross, I. Frydas, and S. Kritas. 2020. 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. Journal of Biological Regulators and Homeostatic Agents 34(2): 327–331. https://doi.org/10.23812/CONTI-E.
14. Kritas, S.K., G. Ronconi, A. Carafa, C.E. Gallenga, R. Ross, and P. Conti. 2020. Mast cells contribute to coronavirus-induced inflammation: new anti-infammatory strategy. Journal of Biological Regulators and Homeostatic Agents 34(1): 9–14. https://doi.org/10.23812/20-Editorial-Kritas.
15. Ronconi, G., G. Teté, S.K. Kritas, C.E. Gallenga, A. Carafa, R. Ross, and P. Conti. 2020. SARS-CoV-2, which induces COVID-19, causes kawasaki-like disease in children: role of pro-inflammatory and anti-inflammatory cytokines. Journal of Biological Regulators and Homeostatic Agents 34(3): 767–773. https://doi.org/10.23812/EDITORIAL-RONCONI-E-59.
16. Dubai Health Authority, https://services.dha.gov.ae/sheryan/wps/portal/home/circular-details?circular RefNo=CIR-2020-00000168&isPublicCircular=1& fromHome=rue, Access date: 13/5/2020.

ISSN: 2776-1010 Volume 4, Issue 2, Feb., 2023

17. Wyllie AL, Fournier J, Casanovas-Massana A, Campbell M, Tokuyama M, Vijayakumar P, et al. Saliva or Nasopharyngeal Swab Specimens for Detection of SARS-CoV-2. N Engl J Med. 2020; 383 (13):1283–6. https://doi.org/10.1056/NEJMc2016359 PMID: 32857487.
18. Sapkota D, Søland TM, Galtung HK, Sand LP, Giannecchini S, To KKW, et al. COVID-19 salivary signature: diagnostic and research opportunities. J Clin Pathol. 2021; 74(6):344. https://doi.org/10.1136/jclinpath-2020-206834 PMID: 32769214.
19. Desai GS, Mathews ST. Saliva as a non-invasive diagnostic tool for inflammation and insulin-resistance. World J Diabetes. 2014; 5(6):730. https://doi.org/10.4239/wjd.v5.i6.730 PMID: 25512775
20. Zhang C-Z, Cheng X-Q, Li J-Y, Zhang P, Yi P, Xu X, et al. Saliva in the diagnosis of diseases. International journal of oral science. 2016; 8(3):133–7. https://doi.org/10.1038/ijos.2016.38 PMID: 27585820
21. Kaufman E, Lamster IB. The diagnostic applications of saliva—a review. Crit Rev Oral Biol Med. 2002;13(2):197–212. https://doi.org/10.1177/154411130201300209 PMID: 12097361
22. Liu J, Li S, Liu J, Liang B, Wang X, Wang H, et al. Longitudinal characteristics of lymphocyte responses and cytokine profiles in the peripheral blood of SARS-CoV-2 infected patients. EBioMedicine. 2020;55:102763. Epub 2020/05/04. https://doi.org/10.1016/j.ebiom.2020.102763 PMID: 32361250.