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Saliva as Alternative Specimen for Measuring Inflammatory Markers Interleukins (IL10, IL-4, and IL-1β) in Association with Disease Severity among COVID-19 Patients

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Abstract

Background: Saliva is a specimen that is easily collected by non-invasive means and does not require well-trained staff; it could be helpful in measuring inflammatory markers to determine COVID-19 severity. The aim of this study was to investigate saliva as an alternative specimen for measuring inflammatory markers IL-10, IL-4, and IL-1β among COVID-19 patients in relation to disease severity.

Methods and Results: This cross-sectional study was conducted among COVID-19 patients in a fever clinic, isolated hotels, and hospitals providing care for positive COVID-19 patients and in public health centers for negative control patients in Jeddah, Saudi Arabia. A total of 151 subjects participated in this study, including 101 patients with COVID-19 and 50 healthy controls. Patients with COVID-19 were categorized according to the severity of their symptoms into mild (n=50) and severe cases (n=51). The salivary concentrations of IL-4, IL-10, and IL-1β were measured using sandwich MyBioSource ELISA Test Kits.

The age of the study population ranged from 19 to 70 years old, with a mean age of 43.3±13.0 years. The distribution of the study population showed that more of the patients were men (65[64.4%]) than women (36[35.6%]) (P=0.004). The frequency of severe infection in men was higher than in women (35[68.6%] and 16[31.4%], respectively, P=0.008). The group of severe cases was significantly older than the group of mild cases (47.9±11.03 years and 38.64±13.82 years, respectively, P=0.0007). The volume of saliva was the smallest in severe COVID-19, compared to mild cases and controls (P=0.0000 in all cases). The salivary levels of IL-4, IL-10, and IL-1β were greater in the severe cases than in mild cases and controls (46.14±11.61 pg/mL, 12.86±1.99 pg/mL, and 27.45±11.47 pg/mL versus 19.31±5.72 pg/mL, 7.96±2.12 pg/mL, and 6.59±1.90 pg/mL, respectively; P=0.0000 in all cases). The salivary levels of IL-4 and IL-10 in mild cases were greater than in controls (19.31±5.72 pg/mL and 7.96±2.12 pg/mL versus 15.30±4.36 pg/mL and 6.02±0.89 pg/mL, respectively; P=0.0329 and P=0.0000, respectively), but salivary IL-1β levels in mild cases did not differ from controls (6.59±1.90 pg/mL vs. 6.03±2.28 pg/mL, P=0.9129).

Conclusion: Saliva could be used as an alternative sample in measuring IL-10, IL-4, and IL-1β with the suggestion of using IL-10 and IL-4 as markers for predicting disease severity.(**International Journal of Biomedicine. 2023;13(2):245-249.**)

Keywords: COVID-19 • saliva • IL-4 • IL-10 • IL-1β

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Introduction

The spectrum of COVID-19 clinical manifestation can range from asymptomatic infection to severe pneumonia, followed by multisystem failure that may lead to death. Inflammatory immune response influences the pathogenesis and clinical expression of COVID-19.[1,2] Cytokine storms can cause a severe clinical complication known as acute respiratory distress syndrome (ARDS). ARDS is induced by an excessive immune response rather than the viral load.[3] Researchers have revealed the association between interleukins and disease severity.[4] Interleukin (IL)-10 is an inflammatory marker predicting disease severity.[5] IL-10 plays an important role in regulating inflammatory response by reducing the damage caused by inflammatory cytokines. It also suppresses the expression of these cytokines and has an important role in controlling the immune response against the virus.[6] Studies have shown that the concentration of serum interleukins is highly increased in cases ranging from severe and critical to mild.[7-11] It has been shown that levels of IL-10 and IL-4 are elevated in COVID-19 patients, compared to healthy individuals, and the level of IL-β has been elevated in non-survivors, compared to survivors. These findings suggest that interleukins are related to disease severity and prognosis.[12]

The effect of the recent COVID-19 pandemic on communities across the world is acute. There is a persistent need for non-invasive, easily self-collected specimens that do not require experienced staff to diagnose and measure inflammatory markers to determine disease severity. Saliva is a specimen that is easily collected by non-invasive means and does not require well-trained staff; it could be helpful in measuring inflammatory markers to determine COVID-19 severity.[13,14] Slavish et al.[15] determined the reliability of saliva specimens in measuring inflammatory markers, including IL-1β, TNF-α, IL-6, IL-2, IL-4, IL-10, IL-12, and CRP, in response to stress across multiple studies. Galhardo et al.[16] investigated salivary inflammatory markers, including IL-6, in hospitalized sepsis patients. They found it to be a useful specimen for diagnosing such a condition. Saliva has been used as a specimen for the detection of the SARS-CoV-2 virus. According to our knowledge, there are no published data about measuring inflammatory markers in saliva among COVID-19 patients.

The aim of this study was to investigate saliva as an alternative specimen for measuring inflammatory markers IL-10, IL-4, and IL-1β among COVID-19 patients in relation to disease severity.

Materials and Methods

This cross-sectional study was conducted among COVID-19 patients in a fever clinic (Tetamman Clinics), isolated hotels, and hospitals providing care for positive COVID-19 patients and in public health centers for negative control patients in Jeddah, Saudi Arabia. Ethical approval was obtained from the Ministry of Health (Saudi Arabia). Formal consent was obtained from participants.

A total of 151 subjects participated in this study, including 101 patients with COVID-19 and 50 healthy controls. Patients with COVID-19 were categorized according to the severity of their symptoms into mild (n=50) and severe cases (n=51). Open-Epi provided the statistics for sample size and power calculations.

Saliva Collection and the Measurement of the Salivary Levels of IL-4, IL-10, and IL-1β

Subjects were given an appointment, preferably between 9 and 11 a.m. They were requested to refrain from eating, drinking, or using oral hygiene products for at least one hour before sample collection. Subjects were asked to rinse their mouth with water three times. Five minutes later, they spit into a Falcon tube kept on ice. Subjects were reminded not to cough out mucus. Saliva was collected for 30 minutes. Salivary samples were centrifuged at 2,600g for 15 min at 4°C. The supernatant was collected and frozen within 30 min from the time of collection.

The salivary concentrations of IL-4, IL-10, and IL-1β were measured using sandwich MyBioSource ELISA Test Kits (https://www.mybiosource.com/elisa-kits) according to the manufacturer's manual. These kits are based on sandwich enzyme-linked immunosorbent assay technology. Standard curves were drawn to obtain the concentration of IL-4, IL-10, and IL-1β in samples.

Statistical analysis was performed using statistical software package SPSS version 25.0 (SPSS Inc, Armonk, NY: IBM Corp). For descriptive analysis, results are presented as mean (M) ± standard deviation (SD). Multiple comparisons were performed with one-way ANOVA with Tukey's pairwise comparisons. Group comparisons with respect to categorical variables are performed using chi-square tests. ROC curves were drawn to evaluate the ability of variables to predict disease severity. A probability value of $P<0.05$ was considered statistically significant.

Results

The distribution of the study population showed that more of the patients were men (65[64.4%]) than women (36[35.6%]) ($P=0.004$). The frequency of severe infection in men was higher than in women (35[68.6%] and 16[31.4%], respectively, $P=0.008$). The frequency of infected men and women among mild cases was 30(60%) and 20(40%), respectively, without statistically significant differences ($P=0.157$) (Table 1).

The age of the study population ranged from 19 to 70 years old, with a mean age of 43.3±13.0 years. The group of severe cases was significantly older than the group of mild cases (47.9±11.03 years and 38.64±13.82 years, respectively, $P=0.0007$) (Table 2).

The volume of saliva significantly differed between COVID-19 patients and the control group ($P=0.0000$), and it was the smallest in severe COVID-19, compared to mild cases and controls ($P=0.0000$ in all cases) (Table 2).

The salivary levels of IL-4, IL-10, and IL-1β were greater in the severe cases than in mild cases and controls (46.14±11.61 pg/mL, 12.86±1.99 pg/mL, and 27.45±11.47 pg/mL versus 19.31±5.72 pg/mL, 7.96±2.12 pg/mL, and 6.59±1.90 pg/mL, respectively; $P=0.0000$ in all cases). The

salivary levels of IL-4 and IL-10 in mild cases were greater than in controls (19.31±5.72 pg/mL and 7.96±2.12 pg/mL versus 15.30±4.36 pg/mL and 6.02±0.89 pg/mL, respectively; *P*=0.0329 and *P*=0.0000, respectively), but salivary IL-1β levels in mild cases did not differ from controls (6.59±1.90 pg/mL vs. 6.03±2.28 pg/mL, *P*=0.9129) (Table 2).

Table 1.

Gender and disease severity among COVID-19 patients.

	Male	Female	P-value
Mild cases	30 (60%)	20 (40%)	0.157
Sever cases	35 (68.6%)	16 (31.4%)	0.008
Total number	65 (64.4%)	36 (35.6%)	0.004

Table 2.

The volume of saliva and inflammatory markers among COVID-19 patients in relation to disease severity.

Variable	Group	Mean ± SD	Statistics
Age, years	(1) Control	36.16 ± 12.91	P=0.0000 P_{1-2}=0.5799, P_{1-3}=0.0000 P_{2-3}=0.0007
	(2) Mild	38.64 ±13.28	
	(3) Severe	47.94 ± 11.03	
Salivary volume, ml	(1) Control	3.70 ± 0.75	P=0.0000 P_{1-2}=0.2953, P_{1-3}=0.0000 P_{2-3}=0.0000
	(2) Mild	3.46 ± 0.98	
	(3) Severe	2.4 1± 0.64	
IL-4, pg/mL	(1) Control	15.30 ± 4.36	P=0.0000 P_{1-2}=0.0329, P_{1-3}=0.0000 P_{2-3}=0.0000
	(2) Mild	19.31 ± 5.72	
	(3) Severe	46.14 ± 11.61	
IL-10, pg/mL	(1) Control	6.02 ± 0.89	P=0.0000 P_{1-2}=0.0000, P_{1-3}=0.0000 P_{2-3}=0.0000
	(2) Mild	7.96 ± 2.12	
	(3) Severe	12.86 ± 1.99	
IL-1β, pg/mL	(1) Control	6.03 ± 2.28	P=0.0000 P_{1-2}=0.9129, P_{1-3}=0.0000 P_{2-3}=0.0000
	(2) Mild	6.59 ± 1.90	
	(3) Severe	27.45 ± 11.47	

Analysis of ROC curves for severe COVID-19 patients showed an excellent area under the curve for all cytokines and salivary volume (Figures 1 and 2). For IL-10, it was 97.3% with the best cutoff (≥10.11); for IL-1B, it was 100% with the best cutoff (≥11.35); for IL-4 - 98.7% with the best cutoff (≥18.25); and for saliva it was 85.0%, with best cutoff (≤3.35).

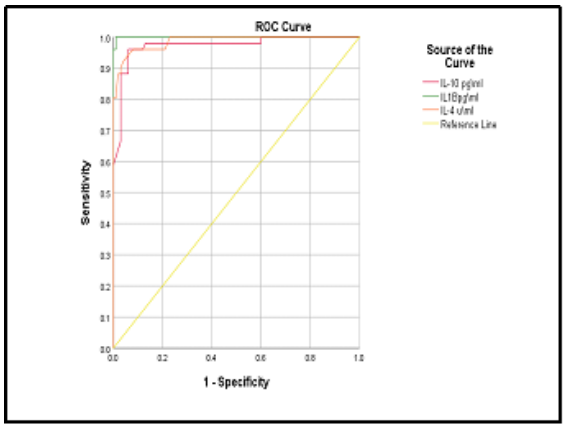

Fig. 1. *ROC curve for the performance of IL-10, IL-4, and IL-1β among severe COVID-19 patients.*

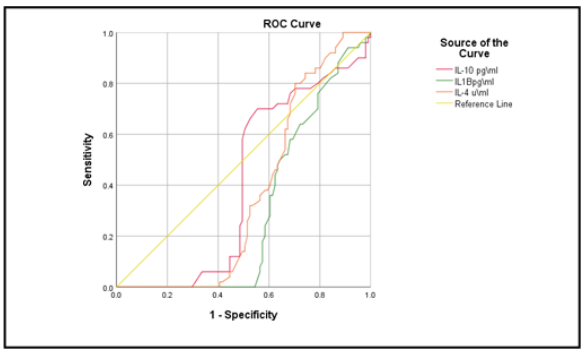

Fig. 2. *ROC curve for the performance of IL-10, IL-4, and IL-1β among mild COVID-19 patients.*

Discussion

This cross-sectional study showed that the percentage of infected men was significantly higher than that of women (65[64.4%]) vs. (36[35.6%]), *P*=0.004), consistent with the finding of Jin et al.[17] The frequency of infected males with severe cases (68.6%) was also significantly higher (*P*=0.008) than females (31.4%), so gender could be a risk factor in exacerbating infection and possibly increasing the mortality rate, as men showed more severe clinical symptoms than women.[17] This susceptibility may be due to high levels of ACE2 in males, which acts as a viral receptor.[18]

The mean age of the study population was 43.3 years and this finding is consistent with study by Abohamr et al.,[19] who examined 768 COVID-19 patients with a mean age of 46.36±13.7 years. The age group of severe cases is significantly older than that of mild cases. Another study reported that older patients tend to have relatively more severe clinical infections and poorer clinical outcomes associated with COVID-19 than do younger patients. Elderly patients aged 65 and older were at a much higher risk of developing severe or critical illness than other age groups.[20]

The volume of salvia among COVID-19 patients was significantly less than among healthy controls and significantly less among severe COVID-19 than among mild COVID-19

cases (P=0.000 in all cases). This finding is consistent with previous studies that report signs of dry mouth among COVID-19 patients.[21-23]

Immune responses have been shown to be involved in the initiation and development of COVID-19. Excessive stimulation of this response results in a cytokine storm that leads to poor prognosis in COVID-19 patients.[7,24,25] We found that the concentration of IL-4, IL-1β, and IL10 was significantly higher in COVID-19 patients than in healthy controls, consistent with previous reports.[5,12,26] The concentration of IL-4, IL-1β, and IL10 in this study was significantly higher among severe COVID-19 cases than among mild cases, which aligns with previous studies.[7-11] IL-10 is a predictor of disease severity. It is highly elevated among COVID-19 patients with severe disease compared, to mild cases and healthy controls. Also, IL-10 was elevated among mild cases, compared to healthy controls, and our finding aligns with several reports.[5,12] Similar changes in the salivary levels were found for IL-4, which disagreed with the results of Han et al.,[5] a discrepancy that may be attributed to the difference in sample size. No significant differences between mild cases and controls in the concentration of IL-1β in our study are like the finding of the meta-analysis by Chang et al.[12]

In conclusion, saliva could be used as an alternative sample in measuring IL-10, IL-4, and IL-1β with the suggestion of using IL-10 and IL-4 as markers for predicting disease severity.

Competing Interests

The authors declare that they have no competing interests.

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