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Original Article

Tracing Some Salivary Immune Elements in Iraqi SARS-2 Patients

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Abstract

Saliva is one of the most significant components in maintaining oral homeostasis and symbiosis. It contains antimicrobial proteins and peptides, such as mucins, lactoferrin, lysozyme, lactoperoxidase, Catherine, statins, and antibodies (secretory immunoglobin A [sIgA]). Early defenses against respiratory infections rely heavily on mucosal immunity, especially secretory sIgA, which has several features and functions that make it suitable for mucosal defense. Salivary testing has been utilized to define mucosal immune responses to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection. Lysozyme has muramidase, with antimicrobial activity, and high concentrations in body fluids, such as saliva and tear. This research aimed to offer an update on how saliva components suppress viral infection and sustain health. A total of 50 individuals, including 30 SARS-2 patients and 20 non-infected subjects, in the age range of 32-54 years were enrolled in this study. Saliva specimens were obtained from polymerase chain reaction (PCR)-confirmed coronavirus disease 2019 (COVID-19) patients and non-infected participants. To collect saliva, the subjects were advised to swirl water over their lips three times, and 5.0 ml of saliva was collected. Samples were centrifuged at 800 x g for 10 min. Saliva was diluted at 1:2,000 with 1 × Diluent N. The immunoglobulin A (IgA) titer in saliva was detected. A spectrophotometer was used to measure the solution's change in absorbance at 550 nm. Measurements (salivary IgA and lysozyme) were made after 7, 30, and 60 days of confirmatory PCR COVID-19 test. The mean scores of salivary IgA levels were obtained at 17.85, 15.26, and 10.73 mg/dl in patients and 9.53, 10.33, and 9.21 mg/dl in healthy individuals after 7, 30, and 60 days, respectively. The salivary lysozyme activity levels in SARS-2 patients compared to controls were 9.7, 7.3, and 4.2 mg/dl versus 2.9, 3.4, and 3.77 mg/dl, respectively. The salivary IgA level was significantly higher in patients of a confirmatory test for COVID-19 compared to healthy individuals.

Keywords: COVID-19, IgA, Lysozyme, SARS-2

1. Introduction

The mucosal immune system protects against viral respiratory illnesses and is vital in both innate and adaptive immunity. Sensor cells, including epithelial cells, macrophages, dendritic cells, and mast cells, respond quickly to pathogens. When these cells detect a pathogen, they initiate innate responses that involve the production of reactive oxygen species and antimicrobial peptides, as well as mucociliary clearance. A large viral inoculum can activate the adaptive immune system of the mucosa, mediated in part by dendritic cells that protrude through the epithelium (1, 2).

The production of antibodies against the pathogen, including immunoglobulin A (IgA), which is the antibody generated in greater amounts and is critical for mucosal immunity, is a component of this adaptive response. Secretory IgA (sIgA) is vital in mucosal

immunity. Immunological exclusion prevents infections from entering host cells by competing for host-cell ligands that stimulate viral entry. Agglutination and protection of microbial adhesions by sIgA for subsequent removal by ciliary activity are two additional functions of the sIgA in viral clearance. The coronavirus spike protein can be neutralized by sIgA antibodies in the case of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), preventing its interaction with the host angiotensin-converting enzyme-2 receptor, or the coronavirus nucleocapsid protein can be bound by sIgA antibodies in the case of SARSr-CoV (3, 4).

Early detection of IgA antibodies in coronavirus disease 2019 (COVID-19) patients may occur as early as 2 days following the beginning of symptoms according to preliminary findings (versus 5 days for either immunoglobulin M or immunoglobulin G). The study of IgA, especially mucosal IgA, in COVID-19 has been delayed. The first antibody to respond to SARS-CoV-2 infection is IgA. The mucosa-specificity of IgA makes it a good target for public health efforts. In comparison to other sample types, salivary testing has been identified by several organizations as desirable because of the simplicity of collecting secreted antibody sIgA (5, 6).

This research was designed to offer an update on how saliva components suppress viral infection and sustain health.

2. Materials and Methods

2.1. Study Design

This study was conducted on 50 polymerase chain reaction (PCR) confirmed individuals, including 30 patients with SARS2 and 20 uninfected subjects as a control, in the same way from March to May 2021. Saliva specimens were obtained from PCR-confirmed COVID-19 patients and non-infected participants. To collect saliva, the subjects were advised to swirl water over their lips three times, and 5.0 ml of saliva was collected. Samples were centrifuged at 800 x g for 10 min. Saliva was diluted at 1:2,000 with 1× Diluent N. The IgA titer in saliva was detected according to Abcam company protocol (Sandwich quantitative enzyme-linked immunosorbent assay, Cat. Number ab137980). The lysozyme assay was performed as previously described by Wang, Riegger (7). A spectrophotometer was used to measure the solution change in absorbance at 550 nm.

2.2. Demographical Data

2.2.1 Age Distribution of Patients with SARS-2 and Controls

The mean age scores of the participants in the COVID-19 patients and control groups were estimated at 40.97 (range of 39-54) and 35.93 (range of 32-43) years (Table 1).

Table 1. Demographical information of the enrolled COVID-19 patients and controls

Studied groups	n	Mean age (years)	Std. deviation	Std. error	Range*	
					Min	Max
Healthy control	20	35.93	9.38	0.94	32	43
COVID-19 patients	30	40.97	11.35	1.72	39	54
Total	50					
Gender		**Male**	**Female**		**Ratio**	
Number		17	13		1:1.31	
Percentage%		56.67%	43.33%			
Min		39	41			
Max		51	54			

2.2.2. Gender Distribution of Patients with SARS-2

As presented in table 1, the percentage of males with SARS-2 was a little bit higher than the percentage of females (56.67% vs 43.33%) with a ratio of 1:1.31.

2.3. Statistical Analysis

The collected data were analyzed in SPSS software (version 21). Quantitative variables were described by numbers, percentages, ratios, and interquartile range. The comparison of qualitative variables was conducted using Pearson's chi-square test.

3. Results and Discussion

3.1. Salivary IgA Levels

The mean scores of IgA concentration in the saliva of SARS-2 patients after 7, 30, and 60 days were obtained at 17.85, 15.26, and 10.73 mg/dl, respectively. In healthy individuals, the mean concentrations after 7,

30, and 60 days were calculated at 9.53, 10.33, and 9.21 mg/dl, respectively. As summarized in table 2, there were significant differences (P<0.05) in the mean IgA concentrations of patients after 7, 30, and 60 days, while no significant differences (P>0.05) were observed in the mean IgA concentrations of controls (2). However, there were highly significant differences (P<0.01) when comparing the mean of SARS-2 patients and healthy individuals after 7 and 30 days, while there were non-significant differences (P>0.05) after 60 days in this regard.

3.2. Lysozyme activity concentration in COVID-19 patient's saliva

The mean values of salivary lysozyme level of SARS-2 patients after 7, 30, and 60 days of PCR COVID-19 respectively. In healthy individuals, the mean scores of lysozyme concentration after 7, 30, and 60 days of PCR COVID-19 confirmation test were estimated at 2.9, 3.4, and 3.77 mg/dl, respectively. There were significant differences (P<0.05) between mean lysozyme concentrations of patients after 7, 30, and 60 days of SARS-2 confirmation, while there were non-significant differences (P>0.05) in the mean of control individuals after 7, 30, and 60 days. However, highly significant differences (P=0.002 and P=0.01) were observed between the mean of SARS-2 patients and healthy individuals after 7 and 30 days of the SARS-2 confirmatory test, respectively. However, there were non-significant differences (P=0.097) after 60 days between the means of COVID-19 patients and healthy controls (Table 3).

Table 2. Salivary IgA concentrations of SARS-2 patients

Group	After 7 days (IgA con. mean±SD) mg/dl	After 30 days (IgA con. mean±SD) mg/dl	After 60 days (IgA con. mean±SD) mg/dl	Pearson's chi-square (*P*-value)
Patients	17.85±3.21	15.26±1.91	10.73±3.77	0.03 Sig (P<0.05)
Control	9.53±0.37	10.33±1.24	9.21±2.11	0.21 non-Sig (P>0.05)
Pearson's chi-square (*P*-value)	0.002 highly Sig (P<0.01)	0.01 highly Sig (P=0.01)	0.067 non-Sig (P>0.05)	

Table 3. Salivary lysozyme concentration in COVID-19 patients

Group	After 7 days (mean±SD) mg/dl	After 30 days (mean±SD) mg/dl	After 60 days (mean±SD) mg/dl	Pearson's chi-square (*P*-value)
Patients	9.7±4.33	7.3±3.72	4.2±2.27	0.04 Sig (P<0.05)
Control	2.9±0.98	3.4±1.25	3.77±1.13	0.091 non-Sig (P>0.05)
Pearson's chi-square (*P*-value)	0.002 highly Sig (P<0.01)	0.01 highly Sig (P=0.01)	0.097 non-Sig (P>0.05)	

Mucosal immunity is greatly aided by IgA. The location of pathogen entrance is the most critical immunoglobulin for combating pathogenic pathogens in the respiratory and digestive systems. Secretory IgA can prevent SARS-CoV-2 from binding to epithelial cells by acting as an immunological barrier (6, 8).

It is conceivable to employ IgA detection as an early diagnostic marker for respiratory infections since IgA levels in the respiratory mucosa may be evaluated in saliva and tears (9). Samples from positive subjects showed elevated salivary IgA 7, 30 days after COVID-19 confirmation. Our findings suggested that salivary confirmation were calculated at 9.7, 7.3, and 4.2 mg/dl, IgA might have a function in the immune system beyond individual patient testing, measuring population infection rates, and assisting vaccine development and deployment.

Patients, on the other hand, had greater salivary levels of IgA than healthy persons. During the first 7 days, it was found that the number of participants with measurable levels of salivary IgA began to decrease. The drop in viral load might explain our findings on the progressive decline in salivary IgA content after 30 and 60 days.

B cells produce IgA in response to T-helper 2 (Th2) secretions, particularly interleukin 5. Regulatory T

(Treg) cells, on the other hand, produce transforming growth factor-β, which inhibits Th2 cell development. According to the findings of this study, there might be a switch in Th2 and Treg cell responses, which might explain the time-dependent variations in salivary IgA levels (10, 11).

A possible explanation for the elevated serum lysozyme levels is enhanced granulocyte turnover as a result of viral infection. Lysozyme is thought to have an indirect effect on infectious pathogens via a mechanism other than microbial lysis (12). The results of a study by Gordon, Douglas (13) indicated that lysozyme, a product of inflammatory cells, worked in a negative feedback mechanism to control the inflammatory response.

Based on the findings of the current research, it is suggested to add salivary IgA tests, which may serve in the early detection of COVID19 patients and verification of their immunocompetence in this regard with greater specificity. According to the results of this study, the salivary IgA level was significantly greater in patients who had a confirming test for COVID-19 than in healthy people. Increased granulocyte turnover due to viral infection of the oral cavity might be linked to increased lysozyme activity in patients.

Authors' Contribution

Study concept and design: S. Q. M.
Acquisition of data: M. M. A.
Analysis and interpretation of data: M. M. M.
Drafting of the manuscript: M. M. M.
Critical revision of the manuscript for important intellectual content: M. M. M. and M. M. A.
Statistical analysis: S. Q. M.
Administrative, technical, and material support: M. M. M.

Ethics

Ethical approval for the study was obtained from the Al-Muthanna University, Al-Muthanna, Iraq.

Conflict of Interest

The authors declare that they have no conflict of interest.

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