Original Article

Immunological Study of Orthodontically Treated Patients Recovering from COVID-19 in Babylon Province, Iraq

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Abstract

Background and Objectives: This study aimed to examine the levels of the toll-like receptors TLR4 and TLR2 in the blood and saliva of patients with coronavirus disease-2019 (COVID-19) receiving orthodontic care in Babylon Province. **Materials and Methods:** Sixty serum and saliva samples were collected and divided into three groups of 20 patients: patients with COVID-19 who did not receive orthodontic treatment (group 1); patients with COVID-19 who received orthodontic treatment (group 2); and patients with COVID-19 who received orthodontic treatment and recovered (group 3). Thirty samples of serum and saliva from healthy outpatient clinics comprised the control group. Enzyme-linked immunosorbent assay was used to determine the result. **Results:** TLR4 levels in saliva increased in all experimental groups (1–3) as compared with the control group. However, only COVID-19 patients and orthodontic patients in group 2 had elevated blood levels of TLR4, whereas the levels were reduced in the other two groups (1 and 3). All experimental groups showed an increase in TLR2 levels in saliva relative to the control group. In the first and second experimental groups, serum TLR2 concentrations increased dramatically, whereas they declined in the third group. There was no correlation between TLR4 and TLR2 in either group of patients. The concentrations of TLR4 and TLR2 in saliva were higher in the experimental groups than in the control group.

Keywords: COVID-19, orthodontic patients, toll-like receptor 2, toll-like receptor 4

Introduction

Coronavirus 2 causes a severe acute respiratory illness known as coronavirus disease (SARS-CoV-2). In December 2019, it was discovered in Wuhan, China, and after rapidly spreading throughout the world, it was proclaimed a global pandemic.[1] Coronavirus disease-2019 (COVID-19) is primarily transmitted through direct contact or respiratory droplets. Consequently, rules for healthcare professionals began to modify, which assisted in preventing the spread of COVID-19.[2,3] The majority of people who test positive for COVID-19 either have mild symptoms or none at all. However, some of these individuals also experience heart failure, blood clots, neurological issues, Hepatitis B virus reactivation, and an inflammatory response that increases the risk of acute respiratory distress syndrome.[4-9] The oral cavity's significance in COVID-19 has been contested. Recent research[10] suggests that the oral mucosa may contribute to the pathogenesis and transmission of SARS-CoV-2. The mouth is essential for eating, communicating, and living a meaningful life. The most prevalent oral diseases are dental caries and periodontal diseases, both of which can typically be prevented.[11] Caries is the most common chronic pediatric ailment that lasts into adulthood. According to national data from 2011 to 2014, 32.7% of adults in the United States had untreated tooth decay.[12] During the suspension of orthodontic practice and home quarantine, many orthodontists and patients stayed at home and communicated via online telemedicine

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services or smartphones.[2,13] Due to the patient's inability to attend regular sessions during this long period of dental treatment suspension,[14] orthodontic concerns, such as loose archwires and brackets, could not be swiftly corrected. Toll-like receptor 4 (TLR4) plays a role in the beginning of inflammatory responses, and its overstimulation can lead to hyperinflammation. Several diseases, including ischemia-reperfusion damage, atherosclerosis, hypertension, cancer, neurodegenerative disorders, and neuropsychiatric disorders, have been linked to the onset or progression of TLR4 signaling dysregulation.[15,16] TLR4 is also involved in the activation of the host's immune system in response to bacterial and viral.[17] Pathogens, including bacteria, viruses, fungi, and parasites, are detected by toll-like receptor 2 (TLR2).[18] Neutrophils and monocytes, among other immune cells, include TLR2. Neutrophils are the first cells to arrive at an infected site, identify the pathogen, and activate an immune response. Alternatively, the binding of a specific ligand to monocytes induces coordinated adaptive response activation at TLR2 and TLR4.[19,20] The aim of this study was to examine the amounts of soluble toll-like receptor 2 (sTLR2) and soluble toll-like receptor 4 (sTLR4) in orthodontic patients healing from COVID-19.

Materials and Methods

Study population

Ninety serum and saliva samples from adults in Babylon city who had orthodontic treatment and were between the ages of 18 and 30 years were collected for the current investigation and divided into four groups. These samples were sorted:

Group 1 consisted of 20 patients with COVID-19 (nasopharyngeal swap positive) who did not receive orthodontic treatment.

Group 2 consisted of 20 patients with COVID-19 (nasopharyngeal swap positive) who received orthodontic therapy.

Group 3 consisted of 20 orthodontic-treated patients who have recovered from COVID-19 (nasopharyngeal swap negative) recovered after two weeks.

Group 4 consisted of 30 serum and saliva samples collected from healthy persons in outpatient clinics with nasopharyngeal swap negative (control group both gender and 18–35 age).

Blood and saliva samples

A disposable syringe was used to collect 5 mL of serum, blood, and then centrifuged samples from 60 patients that were divided into three groups (groups 1–3) and 30 blood and saliva samples from healthy people. Using an enzyme-linked immunosorbent assay (ELISA) and a technique called a quantitative sandwich immunoassay with Elabscience kit the amounts of TLR2 and TLR4 in the blood and saliva were measured. Sandwich ELISA tests were used to measure the levels of TLR2 and TLR4 in serum and saliva, and the results were computed using a standard curve. According to the company (Elabscience), this test achieved the following:

1 Before usage, the kit's components were equilibrated at room temperature.
2 The components of the kit were prepared. Sandwich ELISA was the technique used in this kit of ELISA. In this kit, the micro-ELISA plate was precoated with an antibody specific to human TLR2 and TLR4. Samples or standards were combined with the appropriate antibody in the ELISA plate wells. Each microplate was then treated with an Avidin-horseradish peroxidase (HRP) conjugate and a biotinylated detection antibody specific for human TLR2 and TLR4. Washing was used to remove the free parts. The addition of substrate solution to each well was performed. Only the wells containing human TLR2 and TLR4, the bio-tinylated detection antibody, and the Avidin-HRP conjugates had a blue color. By adding a stop solution, which likewise allowed the color to turn yellow, the enzyme-substrate process was terminated. The optical density (OD) was determined by spectrophotometer at a wavelength of 450 ± 2 nm. The OD value correlated with the concentration of human TLR2 and TLR4. The estimation of both TLR2 and TLR4 human concentrations in the samples was performed by matching the OD of the samples to the standard curve.

Statistical analysis

Data were analyzed by using the Statistical Package for Social Sciences software program, version 23.0. The results were presented as mean and standard deviation. An independent *t* test was used to compare the blood and saliva samples from all patient groups with those from the control group. A value of $P< 0.05$ was considered statistically significant.

Ethical approval

The study was conducted in accordance with the ethical principles. It was carried out with patient's verbal and analytical approval before the sample was taken. The study protocol and the subject information and the consent form were reviewed and approved by Babylon University College of Dentistry, a local ethics committee according to document number 102 on November 1, 2022, to get this approval.

Results

The outcomes of this study revealed that the TLR4 in saliva increased in three groups as compared with the control group. Interestingly, TLR4 serum levels were

Table 1: Mean and standard deviation of toll-like receptor 2 and toll-like receptor 4 in patients and control group (group 1)

Parameters	Mean ± SD		*P* Value
	Patients	Control	
TLR2 serum	3.976 ± 2.35	2.876 ± 1.93	0.21
TLR2 saliva	62.913 ± 53.79	164.891 ± 46.25	0.000***
TLR4 serum	293.943 ± 97.55	187.284 ± 133.66	0.04*
TLR4 mucosal*	0.307 ± 0.21	0.134 ± 0.04	0.002**

SD = standard deviation
Significant at $P < 0.05$
*Significant
**Very significant
***Very high significant

Table 2: Mean and standard deviation of toll-like receptor 2 and toll-like receptor 4 in patients and control group (group 2)

Parameters	Mean ± SD		*P* Value
	Patients	Control	
TLR2 systemic	306.677 ± 90.776	2.876 ± 1.93	0.02*
TLR2 mucosal	109.929 ± 94.666	164.891 ± 46.25	0.04*
TLR4 systemic	3.1617 ± 2.08	187.284 ± 133.66	0.7
TLR4 mucosal	0.744 ± 0.721	0.134 ± 0.04	0.001**

SD = standard deviation
Significant at $P < 0.05$
*Significant
**Very significant

Table 3: Mean and standard deviation of toll-like receptor 2 and toll-like receptor 4 in patients and control group (group 3)

Parameters	Mean ± SD		*P* Value
	Patients	Control	
TLR2 systemic	0.02*	2.876 ± 1.93	305.605 ± 113.78
TLR2 mucosal	0.08	164.891 ± 46.25	236.840 ± 165.90
TLR4 systemic	0.08	187.284 ± 133.66	4.230 ± 1.94
TLR4 mucosal	0.006*	0.134 ± 0.04	0.537 ± 0.58

SD = standard deviation
Significant at $P < 0.05$
*Significant

increased only in the second group as compared with the control group, whereas concentrations declined in the other two groups (groups 1 and 3). TLR2 concentrations in saliva were significantly higher in all the three groups (groups 1–3) as compared with the control group. TLR2 serum concentrations increased in the first and second groups relative to the control group, but decreased in the third group, as seen in Tables 1–3.

Discussion

TLR4 is a "pattern recognition receptor" that is essential for the innate immune system. Pathogen-associated molecular patterns (PAMPs) are shared by bacteria, viruses, and other pathogens.[21] During viral infection, "host tissue injury, specific", "damage-associated molecular patterns" such as heat-shock proteins, and "high mobility group box 1" were identified and generated by dead or lytic cells.[22] TLR4 is expressed on the cell surfaces of immune cells such as macrophages and dendritic cells, and this expression aids in the management of acute inflammation. It is also expressed on the cell surface of some populations of tissue-resident cells for cell defense against infection and/or to alter their fibrotic phenotype after tissue damage.[23,24]

TLR2 is unique in that it forms a heterodimer with TLR1 or TLR6 to detect and respond to microbial cell wall components such as lipoteichoic acid, peptidoglycan, and lipoproteins or lipopeptides.[25,26] Sarah *et al.*[27] reported that TLR2 expression was greater in gingivitis samples. TLR2 identifies the aforementioned PAMPs that are frequent in gingivitis. Furthermore, Hajishengallis *et al.*[28] discovered that *Porphyromonas gingivalis* acting through TLR2 can activate a different inside-out signaling pathway than lipopolysaccharide acting through TLR4, as seen in chronic periodontitis. This current investigation found a high concentration of TLR-2 when compared to the control group, which is consistent with Zhao *et al.*,[29] who found that the level of sTLR-2 in saliva with active caries was substantially greater (29.5 ± 3 pg/mL) than in saliva devoid of caries (24.8 ± 0.6 pg/m). Previous research has shown that TLR signaling in host cells triggers cytokine release.[30] This finding was compatible with the findings obtained by several authors worldwide,[31-32] who discovered that the healthy group had significantly lower values for all clinical measures ($P = 0.05$). TLR4 concentrations in saliva were significantly higher in chronic periodontitis patients ($P = 0.05$) than in the control group [Tables 1–3]. The second and third groups had higher serum TLR2 concentrations than the control group, while the first group had lower serum TLR2 concentrations. TLR4 concentration was only high in the first group when compared to the control group, but low in the second and third groups when compared with the control group.

Conclusion

Three patient groups had higher TLR2 and TLR4 concentrations in their saliva as compared with the control group, TLR4 concentrations increase in COVID-19 patients and orthodontic patients in group 2 only; however, the levels reduced in the other two groups (groups 1 and 3). TLR2 concentrations in serum increased in the first and second experimental groups, whereas TLR2 concentrations decreased in the third group.

Data availability

All the data that support the study results are available from the corresponding author (Basma Abdel Khaleq Eidan, e-mail: basma.abid@mustaqbal-college.edu.iq) upon request.

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Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Zhu N, Zhang D, Wang W, Li X, Yang B, Song J, *et al*. A novel coronavirus from patients with pneumonia in China, 2019. N Engl J Med 2020;382:727-33.
2. Meng L, Hua F, Bian Z. Coronavirus disease 2019 (COVID-19): Emerging and future challenges for dental and oral medicine. J Dent Res 2020;99:481-7.
3. Ather A, Patel B, Ruparel NB, Diogenes A, Hargreaves KM. Coronavirus disease 19 (COVID-19): Implications for clinical dental care. J Endodont 2020;46:584-95.
4. Zhou F, Ting Y, Ronghui D, Guohui F, Ying L, Zhibo L, *et al*. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: A retrospective cohort study. Lancet 2020;395:1054-62.
5. Akkaif MA, Sha'aban A, Cesaro A, Jaber AAS, Vergara A, Yunusa I, *et al*. The impact of SARS-CoV-2 treatment on the cardiovascular system: An updated review. Inflammopharmacology 2022;30:1143-51.
6. Akkaif MA, Bitar AN, Al-Kaif LAIK, Daud NAA, Sha'aban A, Noor DAM, *et al*. The management of myocardial injury related to SARS-CoV-2 pneumonia. J Cardiovasc Dev Dis 2022;9:307.
7. Al-Kaif LA, Al-Asadi HA, Al-Khafaji YA, Al-Mammori RT, Mezher HA, Kazem WM, *et al*. Association between the levels of Bradykinin and viral infection in patient suffering from respiratory infection, renal transplant, and renal failure. Ann Trop Med Public Health 2020;23:S12.
8. Al-Kaif LAIK, Al-Saadi MAK, Al-Charrakh AH. Coinfection of COVID19 and viral hepatitis: A rapid review. Int J Health Sci 2022;6:4976-87.
9. Al-Kaif LAIK, Al-Saadi MAK, Al-Charrakh AH. Effect of SARS-COV-2 infection on HBV-infected patients: Reactivation. Med J Babylon 2022;19:736-46.
10. Xu H, Zhong L, Deng J, Peng J, Dan H, Zeng X, *et al*. High expression of ACE2 receptor of 2019-nCoV on the epithelial cells of oral mucosa. Int J Oral Sci 2020;12:1-5.
11. Jepsen S, Blanco J, Buchalla W, Carvalho JC, Dietrich T, Dörfer C, *et al*. Prevention and control of dental caries and periodontal diseases at individual and population level: Consensus report of group 3 of joint EFP/ORCA workshop on the boundaries between caries and periodontal diseases. J Clin Periodontol 2017;44:S85-93.
12. Kaye EA, Sohn W, Garcia RI. The healthy eating index and coronal dental caries in US adults: National Health and Nutrition Examination Survey 2011–2014. J Am Dent Assoc 2020;151:78-86.
13. Machado RA, de Souza NL, Oliveira RM, Júnior HM, Bonan PRF. Social media and telemedicine for oral diagnosis and counselling in the COVID-19 era. Oral Oncol 2020;105:104685.
14. Suri S, Vandersluis YR, Kochhar AS, Bhasin R, Abdallah MN. Clinical orthodontic management during the COVID-19 pandemic. Angle Orthodont 2020;90:473-84.
15. Kuzmich NN, Sivak KV, Chubarev VN, Porozov YB, Savateeva-Lyubimova TN, Peri F. TLR4 signaling pathway modulators as potential therapeutics in inflammation and sepsis. Vaccines 2017;5:34.
16. Biancardi VC, Bomfim GF, Reis WL, Al-Gassimi S, Nunes KP. The interplay between angiotensin II, TLR4 and hypertension. Pharmacol Res 2017;120:88-96.
17. Mukherjee S, Karmakar S, Babu SPS. TLR2 and TLR4 mediated host immune responses in major infectious diseases: A review. Braz J Infect Dis 2016;20:193-204.
18. Oliveira-Nascimento L, Massari P, Wetzler LM. The role of TLR2 in infection and immunity. Front Immunol 2012;3:79.
19. O'Mahony DS, Pham U, Iyer R, Hawn TR, Liles WC. Differential constitutive and cytokine-modulated expression of human toll-like receptors in primary neutrophils, monocytes, and macrophages. Int J Med Sci 2008;5:1-8
20. Blasius AL, Beutler B. Intracellular toll-like receptors. Immunity 2010;32:305-15.
21. Mogensen TH, Paludan SR. Reading the viral signature by toll-like receptors and other pattern recognition receptors. J Mol Med 2005;83:180-92.
22. de Kleijn D, Pasterkamp G. Toll-like receptors in cardiovascular diseases. Cardiovasc Res 2003;60:58-67.
23. Turner NA. Inflammatory and fibrotic responses of cardiac fibroblasts to myocardial damage associated molecular patterns (DAMPs). J Mol Cell Cardiol 2016;94:189-200.
24. Rosadini CV, Kagan JC. Early innate immune responses to bacterial LPS. Curr Opin Immunol 2017;44:14-9.
25. MacRedmond R, Greene C, Taggart CC, McElvaney N, O'Neill S. Respiratory epithelial cells require toll-like receptor 4 for induction of human β-defensin 2 by lipopolysaccharide. Respir Res 2005;6:1-11.
26. Hans M, Hans VM. Toll-like receptors and their dual role in periodontitis: A review. J Oral Sci 2011;53:263-71.
27. Sarah SM, Tamilselvan S, Kamatchiammal S, Suresh R. Expression of Toll-like receptors 2 and 4 in gingivitis and chronic periodontitis. Indian J Dent Res 2006;17:114.
28. Hajishengallis G, Wang M, Liang S. Induction of distinct TLR2-mediated proinflammatory and proadhesive signaling pathways in response to *Porphyromonas gingivalis* fimbriae. J Immunol 2009;182:6690-6.
29. Zhao A, Blackburn C, Chin J, Srinivasan M. Soluble toll like receptor 2 (TLR-2) is increased in saliva of children with dental caries. BMC Oral Health 2014;14:1-5.
30. Takeda K, Kaisho T, Akira S. Toll-like receptors. Annu Rev Immunol 2003;21:335335-376.
31. Buduneli N, Özçaka O, Nalbantsoy A. Salivary and plasma levels of toll-like receptor 2 and toll-like receptor 4 in chronic periodontitis. J Periodontol 2011;82:878-84.
32. Jebur MH, Hamawandi JA, Al-Hasnawy HH, Abdul-Lateef LA-R, Al-Charrakh AH. COVID-19, history and modern laboratory diagnostic techniques: A rapid review. J Pharm Negative Results 2022;13:321-26.