Effect of Covid-19 vaccine on some immunological salivary biomarkers (sIgA and Interleukine-17)

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Abstract:

**Background:** The most widely used vaccination against SARS-associated coronavirus (SARS-CoV-2) is the Pfizer vaccine, which provides protection against this virus. However, its ability to safeguard the oral cavity is unclear, and neither are the exact immunological biomarker levels it activates.

**Aim of the study:** To investigate the possibility that Pfizer vaccination protects the oral cavity against Covid-19.

**Patients and Methods:** The study group consisted of a total of 70 subjects (30 as the control group They were followed up before being vaccinated as non-vaccinated (maybe previously infected or non-infected or recovered) and 40 participants followed up three weeks after the first dose and one week after the second vaccination. All saliva samples were collected from the individuals in the current study at the medical city hospital in Baghdad from September 2021 to July 2022. The salivary biomarkers sIgA and IL-17 were detected by enzyme-linked immunosorbent assay (ELISA) kits.

**Result:** Secretory IgA levels showed a highly significant difference (p<0.05) in the followed-up group after the first vaccination compared to the non-vaccinated group (controls), however, a non-significant difference in its level was found in the followed-up group after the first vaccination compared to after the second vaccination. In contrast to healthy controls, non-vaccinated participants had greater salivary IL-17 levels. Followed-up participants’ IL-17 levels did not change significantly after the first and second vaccines (P>0.05).

**Conclusion:** The Pfizer vaccine had a minor impact on sIgA because mRNA vaccines protect systemically more than salivary. Nevertheless, the Pfizer vaccine raises IL-17 levels after the first and second doses without triggering cytokine syndrome.

**Keywords:** SARS-CoV-2, Covid-19, Vaccination, Pfizer (BNT162b2), Secretory (sIgA), IL-17.

Introduction:

The COVID-19 virus caused the worst pandemic. It is thought to have started in Wuhan, China in December 2019. The SARS-CoV-2 virus belongs to the family of Coronaviridae and has spike-like glycoproteins encoding S, M, E, and N subunits. However, the S1, RBD, and S2 types are the actual vaccine target as they can damage the respiratory tract and other organs related to the action of angiotensin-converting enzyme 2 (ACE2) receptors in the tongue, mucosa, and salivary glands.1 Viral infections can damage the oral epithelial cells and cause inflammation such as Xerostomia is considered a serious dental risk following COVID-19 infection, as it can cause dental caries, inflammation, fissuring of the lips (cheilitis), ulcerations of the oral mucosa, inflammation of the buccal mucosa and tongue, oral candidiasis, parotid gland enlargement, and salivary gland enlargement.2 This viral infection may be asymptomatic, moderate, or severe causing death. For this reason, long-lasting immunization is needed to avoid repeated attacks.3 The FDA-approved two-dose SARS-CoV-2 mRNA vaccines in December 2020 including BNT162b2/Pfizer and mRNA-1273/Moderna. Phase-3 trials for both vaccines reported high efficacy in preventing symptomatic infections after the second dose. It is well known that antigens and vaccines can trigger B- and T-cell responses, in the form of antibody production ("humoral immunity"), and ("cellular immunity") mediated by T-cell responses including CD4+ helper cell and CD8+ Cytotoxic lymphocytes which inhibit the spread of infectious pathogens by detecting contaminated cells or secreting antiviral cytokines.4 Vaccine-induced immune effectors are antibodies that bind to pathogens.5 Researchers demonstrated antibodies in the saliva including IgG, IgM, and IgA against SARS-CoV-2 spike protein and RBD of SARS-covid-19 that have a potential role against covid-19, in patients in the acute and convalescent stages of the illness. This antibody function neutralizes the virus and protects the host against re-infection by viral pathogens.6,7 The saliva determines the oral environment and is a convenient diagnostic and monitoring indicator of diseases. It contains a number of minerals, stress hormones such as cortisol, proteins, anti-microbial peptides,
lysozymes, and immunoglobulins, particularly the secretory IgA (sIgA) that plays a key role in attacking SARS-CoV-2. Secretory IgA is an essential immunological biomarker that neutralizes the virus and restricts its adhesion to and invasion of epithelial cells. In addition, it can agglutinate and facilitate the clearance of the pathogen including viruses in mucus secretion.11 On the other hand, tissue-resident memory (TRM) T and B cells, mucosa-associated invariant T (MAIT) cells, in addition to the mucosal complement system, interferons (IFNs), and cytokines play a key role in attacking SARS-CoV-2. Cytokines can influence the initiation and maintenance of the immune response.12 Activation of innate immunity triggers cell-mediated immunity virtually, and, simultaneously especially, the Th17 sub-population, to produce CD4+ T cells, produce IL-17A, IL-17F, and IL-21,22, thus Interleukin-17 (IL-17) pro-inflammatory cytokine a pleiotropic play a role in pathogenesis and immune protection.13 It is a critical cytokine for host defense against mucosal infections and inflammations at the oral barrier and plays a prominent pathogenic and therapeutic role in autoimmune and inflammatory illnesses. IL-17-secretion from CD4+ T cells (Th17) was first identified as a significant generator of this cytokine. CD8+ T cells, T cells, ILCs, NK cells, invariant NK T cells, mucosal-associated invariant T cells, mast cells, and Paneth cells may also produce IL-17.14 Although, it has signaling and synergistic effector molecules (IL-6, IL-1β, TNFα), and chemo-attractants (IL-8 & MCP1) predict COVID-19 clinical development and severity. Synergistic IL-17A and IL-6 that polarise Th17 cells cause lung fibrosis and respiratory failure, and the development of edema on the mucosal surfaces of the respiratory tracts of COVID-19 patients may be the result of systemic inflammation that is associated with Th17.15 Furthermore, the Pfizer vaccine was proven to trigger immune responses. There was just a mild innate immune response 1 - 7 days following the first vaccination that has been demonstrated to produce significant frequencies of antigen-specific CD8+ T cell responses.16 Research suggests that an oral or nasal vaccination may suppress SARS-CoV-2 at the mucosal level, inhibiting further transmission through a robust mucosal and systemic immune response. Vaccination of the oral or nasal mucosa may stimulate an immune response that blocks the virus in its tracks.17 Consistent with this concept, it was found as revealed that in infected people, the mucosal IgA response was negatively linked with symptom intensity, being more plentiful in asymptomatic COVID-19 patients, therefore strengthening their function in preventing viral entry into the body.18 The research found minimal levels of sIgA in saliva, but substantial levels of vaccine-induced IgG, in which the mRNA BNT162b2 vaccination elicits a robust systemic immune response by dramatically enhancing neutralizing antibody development in serum, but not in saliva.19 indicating that at least oral mucosal immunity is inadequately stimulated by this vaccination protocol, thereby failing to prevent virus acquisition via this route.

Patients and Methods

The Research Ethics Committee by the University of Baghdad College of Dentistry approved the research on December 2021. This research study was conducted in the Medical City Hospital at the Private Nursing Home outlet for vaccinations against Covid-19, as well as in Baghdad Consultation and Teaching Hospital Complex during the period between 27 September 2021 to July 2022. Saliva samples were collected for a total of 70 participants (30 as the control group They were followed up before being vaccinated as non-vaccinated (maybe previously infected or non-infected or recovered) and 40 participants followed up three weeks after the first dose and one week after the second vaccination. They were the study groups of the current study. The Inclusion criteria for this study included participants between (20-60) years old, of both genders. Exclusion criteria included alcoholics and patients with comorbidities (diabetes mellitus, cardiovascular diseases, chronic kidney disease, chronic lung disease, and immunosuppression), in addition to pregnant women. Also, excluded were patients who did not finish the questionnaire or refused to participate. All saliva samples after collection and storage of their salivary biomarker sIgA, IL-17 were analyzed by Enzyme-linked immunosorbent assay (ELISA) kits. Saliva collection: The Participants in the current study were provided with a special questionnaire from a special case sheet to require personal information including Name, Age, phone number, a past infection with covid-19 or not, chronic disease, oral diseases, Type of vaccine, first vaccine dose, second vaccine dose, date of obtained vaccine, and gender. And sample collected of approximately one - three milliliters of whole unstimulated saliva from the same patient at three different times: Before immunization, three weeks after the first dose of vaccination, and one week later after the second dose of vaccination, and they were instructed to abstain from meals for a half-hour before the saliva sample was obtained to avoid contamination, and to reduce diurnal fluctuations associated with saliva collection.20 all saliva samples were obtained between 9 a.m. and 12 p.m. The saliva sample was transferred into a sterile plain tube and centrifuged for 15 minutes at 3000 rpm. The supernatant was collected in an Eppendorf tube, and all samples were kept in the deep freezer at (-80°C) until analysis.21 Blood collection: Ten µl of whole blood samples were collected using capillary tubes, then two drops of sample buffer were installed, and introduced to the buffer well of the test device, when the blue control band turns red the result was read within 10 minutes.22 IgG and IgM Anti-Coronavirus specific antibodies were tested using rapid screening tests performed for each patient in this study by using the COVID-19 IgG/IgM Rapid Test Kit from Assure
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Statistical analysis

In this investigation, SPSS version 26, and Microsoft Excel 2010 were employed. To evaluate the difference between groups, Statistical tests were thus used. One-way ANOVA post-hoke LSD evaluated differences. And A chi-square statistic is a measure of the difference between the observed and expected frequencies of the outcomes of a set of events or variables.

Table 1: Comparing the age and gender of the two study groups

<table>
<thead>
<tr>
<th>Variables</th>
<th>Groups</th>
<th>Total (70) (100%)</th>
<th>Statistical Test</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>Controls (30) No. (%)</td>
<td>Case (40) No. (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>14 (46.7)</td>
<td>18 (45.0)</td>
<td>32 (45.7)</td>
<td>0.019*</td>
</tr>
<tr>
<td>Male</td>
<td>16 (53.3)</td>
<td>22 (55.0)</td>
<td>38 (54.3)</td>
<td></td>
</tr>
<tr>
<td>Age (year) mean±SD</td>
<td>34.0±9.50</td>
<td>32.8±9.40</td>
<td>33.3±9.40</td>
<td>0.071**</td>
</tr>
</tbody>
</table>

* Chi-square test, ** Student t-test

Table 2 shows that there was a non-significant elevation in the salivary levels of sIgA among the non-vaccinated patients group compared with healthy controls, with the mean±SE being (25.38±0.433), (24.97±0.558), respectively, (P=0.560).

Table 2: sIgA level in non-vaccinated patients and healthy control groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean ±SE</th>
<th>F</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy control (n=30)</td>
<td>24.973 ±0.558</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-vaccinated patients (n=40)</td>
<td>25.381 ±0.433</td>
<td>0.343</td>
<td>NS</td>
</tr>
<tr>
<td>Total (n=70)</td>
<td>25.206 ±0.342</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

df=2, NS= Non-Significant (P>0.05)

Table 3 shows a significant increase in the salivary levels of IL-17 in the non-vaccinated patient group compared to healthy controls with a mean±SE, (208.66±14.824), (133.01±4.998), respectively, (P<0.001).

Table 3: Descriptive statistic of IL-17 level in the Non-vaccinated and Healthy control groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean ±SE</th>
<th>F</th>
<th>p. value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy control (n=30)</td>
<td>133.01 ±4.998</td>
<td></td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Non-vaccinated (n=40)</td>
<td>208.66 ±14.824</td>
<td>21.040**</td>
<td>Sig.</td>
</tr>
<tr>
<td>Total (n=70)</td>
<td>176.24 ±9.7868</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

df=2, ** Significant (P<0.01).

Table 3 shows a significant increase in the salivary levels of IL-17 in the non-vaccinated study group compared to healthy controls with a mean±SE, (208.66 ±14.824), (133.01±4.998), respectively, (P<0.001).

Discussion

Covid-19 is a highly contagious new respiratory virus that spread quickly globally while many governments were unprepared for it. Some countries were able to control the virus, and vaccines were developed within the first year to control the viral spread. One of the first approved vaccines was BNT162b2.23 mRNA
COVID-19 vaccines can trigger effective immune system pathways that lead to providing successful protection against SARS-CoV-2.24 The present investigation found that the control group and the study groups did not vary significantly from one another when it came to age or gender. This may be explained due to the safety of this vaccine's effectiveness for different age groups, whether for young people with a great immunological response to COVID-19 immunization or for older ones who have lower immunity. Jensen et al 25 showed no statistically significant differences between males and females vaccinated by Pfizer-BioNTech. In the current investigation, no significant changes in sIgA levels were found between non-vaccinated patients and healthy controls, besides a substantial reduction in this mucosal antibody after two doses of vaccine. This outcome may be related to the mechanism of vaccine delivery through parenteral routes which stimulate mainly IgG and IgM antibodies and smaller amounts of secretory IgA antibodies which may lead to its low levels in mucosal locations (upper respiratory tract) as indicated by De Magistris 26. This result agrees with Darwich et al 27 who stated that the BNT162b2 vaccine allows for the release of SARS-CoV-2-specific Ig in the saliva which originates from serum back to gingival crevices and then to the saliva and found that SARS-CoV-2-specific Ig both in the saliva and in the plasma is almost completely lost at three months. Sheikh-Mohamed et al 28 found that the levels of antigen-specific IgG and IgA in the saliva were significantly decreased at this time point in comparison to two weeks after the second dosage. According to Mohamed et al 29, saliva is an important biofluid that might potentially provide information on the antibody (Ab) response of the mucosal mucosa to SARS-CoV-2. Antibodies that are present in saliva may have originated in the blood and made their way to the gingival crevicular fluid via the gingival crevices. Despite this, localized Ab responses, such as the production of secretory IgA (sIgA) by the salivary glands, have been shown to be important. Furthermore, Azzi et al 18 reported that following a single dose of vaccination, the serum IgA concentration in seropositive (SP) persons seems to plateau and does not rise after a second dose. After the second dosage, IgA levels in the seronegative (SN) group increased relative to the seropositive group, reaching a greater concentration. This may be explained by the fact that earlier virus exposure may first induce mucosal IgA response, comparable to other viral infections, rather than systemic immunity. This study found that the vaccination protocol inadequately stimulates oral mucosal immunity, leading to a decline in sIgA levels. This is consistent with Sano et al 30 Those who had been infected in the past and who got the vaccine were able to develop a quick and robust anti-spike sIgA response by boosting their mucosal immunity, but those who had not been infected in the past produced just a small amount of mucosal immunity. In contrast to the study by Sundar et al 31, antibodies specific for SARS-CoV-2 spikes have been detected in sufficient quantities in saliva samples, particularly in individuals who had received both doses of immunization and exhibited higher antibody levels than those who had received only one dose. Those with a history of covid-19 infection who were not inoculated had less protection than those who had been exposed to infection after vaccination. Thus, this could be due to some infections, unhealthy dietary choices, and persistent food allergies. Vitamin D, vitamin A, zinc, and glutathione are essential nutrients for maintaining appropriate levels. However, the sample size was small to identify a correlation between individuals' mucosal immunity and vaccine, suggesting that the responses were a recall of immunological memory induced by previous covid-19 infection. Regarding IL-17, the present study showed a significant increase in the salivary levels of IL-17 in the non-vaccinated study group compared with healthy controls. SARS-CoV-2 infection may be a subclinical or mild illness in some, with their immune response following such infection being an exuberant activation of T cells and Th17 cell infiltration leading to an elevation of inflammatory cytokines including IL-17, in addition to specific antibodies which remain for a period of time after the infection, as suggested by Zhang et al 32. As far as we know, there have been no previous studies comparing the levels of IL-17 in the patient’s saliva after infection with covid-19 or vaccination. However, our study was supported by a similar study conducted by Ong et al 33 who found that COVID-19 patients had distinct systemic cytokine profiles compared with healthy controls. The levels of pro-inflammatory T cell-associated cytokines such as IL-17A, IL-12p70, and IL-β, were elevated and increased post-discharge. The current study showed a highly significant difference in IL-17 levels in the non-vaccinated control group compared with that followed-up after the first vaccination, which may be due to the superior antibody response generated after the vaccine. Boilles et al 34 used inactivated SARS-CoV vaccines to replicate Th1/Th2 responses associated with lung immunological disease where Th1 responses produce interferon-gamma (IFN-γ) and IL-12, whereas Th2 reactions yield IL-4. Th17, regulatory T-cell balance in addition to pro-inflammatory CD4+T-cell response, cytotoxic T-lymphocyte activity is affected by self-reactive IL-6 as suggested by Kimura & Kishimoto 35 This explains the present study findings for the followed-up patients after receiving a second vaccination. A similar study was done by Rokni et al 36 who stated that activation of Th17 cells may lead to the release of inflammatory cytokines such as IL-17, which can worsen the inflammatory responses by activating downstream cytokines such as IL-1, IL-6, IL-8, TNF-alpha, and MCP-1. In the presence of IL-6, IL-1, IL-23, and TGF-β, native CD4+ T cells may develop into Th17 cells, as suggested by Jin and Dong 37. In patients with severe COVID-19, the Treg/Th17 cell ratio is low owing to a low number of Treg cells, vitamin A, zinc, and glutathione are essential nutrients for maintaining appropriate levels.
suggesting poor control of pro-inflammatory responses. This balance is also related to the severity of uncontrolled systemic inflammation in Acute lung injury (ALI) and acute respiratory distress syndrome (ARDS). Therefore, dysregulation of the Treg/Th17 cells ratio skewing toward the Th17 phenotype may contribute to uncontrolled cytokine and chemokine cascades in COVID-19 patients, resulting in worsening inflammatory responses and tissue damage, Martonik et al. 38 Moreover, Th17 cells generate IL-17, IL-17F, IL-21, and IL-22. Although the importance of Th17 cells in initial immune responses against infections is well recognized. Accumulating information suggests that the lineage may be crucial for vaccine-induced memory immunological responses against infectious illnesses, Lin et al. 39 Thus, to provide mucosal homeostasis it able to depend on Th17-type immunity, also called type-17, and Th3 immunity, which produces IL-17/IL-22. IL-17 drives neutrophils and tissue healing, whereas IL-22 increases epithelial cell growth and modulates epithelial permeability, mucus, antimicrobial proteins, and complement to preserve barrier integrity. Furthermore, in mucosal tissues, Th17 CD4+ T cells and T cells may persist as effector memory cells. Inducing protective Th17-type responses in a disease or vaccine scenario requires a sophisticated understanding of Th17 subsets and functional responses, Merino et al. 40.

**Conclusion**

Pfizer COVID-19 vaccine seems to have a limited effect on the sIgA because the mRNA vaccine action is related to systemic protection rather than the local salivary protection role in the body. The vaccine seems to promote triggering the level of IL-17 in the body after the first and second doses of immunization.

**Authors’ Declaration:**

We hereby confirm that all the Figures and Tables in the manuscript are mine/ours. Besides, the Figures and images, which are not mine/ours, have been given permission for re-publication attached with the manuscript.-Authors sign on ethical consideration’s approval-Ethical Clearance: The project was approved by the college’s in-house ethics committee of Dentistry, University of Baghdad. according to the code number (project No. 406821).

Conflicts of Interest: None.

**Authors contributions:**

Dhuha M. Ali : MSc student

Ghada I. Taha: Supervisor

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responses are variably induced in response to SARS-CoV-2 mRNA vaccination and are associated with protection against subsequent infection Mucosal immunology. 2022; 1-10.


Effect of Covid-19 vaccine on some immunological salivary Biomarkers
(sIgA and Interleukine-17)

Dhuha M. Ali

Tahawul: واشنط كوفيد-19 لقاح فايزر، الذي يوفر الحماية من هذا الفيروس.

الخلفية: التطعيم الأكثر استخدامًا ضد فيروس كورونا المرتبط بالسارس (SARS-CoV-2) هو لقاح فايزر، الذي يوفر الحماية من هذا الفيروس. ومع ذلك، فإن قدرته على حماية تجويف الفم غير واضحة، ولا مستويات المؤشرات الحيوية المناعية الدقيقة التي ينشطها.

الأهداف: الكشف بما إذا كان التطعيم بلقاح فايزر يحمي تجويف الفم أم لا.

المريض والطريقة: تكونت مجموعة الدراسة من إجمالي 70 شخصًا (30 شخصًا كمجموعة ضابطة تمت متابعتهم قبل التطعيم على أنهم غير ملقحين (ربما مصابين سابقًا أو غير مصابين أو تعافوا) ومتابعة 40 مشاركًا بعد ثلاثة أسابيع الجرعة الأولى وبعد أسبوع من التطعيم الثاني. تم جمع عينات اللعاب sIgA من فئات مجموعة الدراسة في مستشفى المدينة الطبية في بغداد من سبتمبر 2021 إلى يوليو 2022. تم الكشف عن المؤشرات الحيوية اللعابية (ELISA).

النتائج: أظهرت مستويات إفراز IgA للفيروس (P<0.05) في مجموعة المتابعة بعد التطعيم الأول مقارنة بالمجموعة غير الملقحة (مجموعة الضابطة). ومع ذلك، وجد اختلاف غير معنوي في مستوى IgA في مجموعة المتابعة بعد التطعيم الأول مقارنة بعد التطعيم الثاني. على عكس الضوابط الصحية، كان لدى المشاركين غير الملقحين مستويات أعلى من IL-17 للمناعة ضعيفة. تم تغذير مستويات IL-17 للمناعة ضعيفة في المشاركين المتابعين بشكل ملحوظ بعد التطعيم الأول والثاني (P<0.05).

الخلاصة: لقاح فايزر لقياح كوفيد-19 لا يتأثر طفيفًا بالجرعة الأولى والثانية دون التسبب في متلازمة السيتوكين. 

الكلمات المفتاحية: كوفيد-19، التطعيم فايزر (BNT162b2)، الأفراز (sIgA)، IL-17.