Antibody Response to BNT162b2 mRNA Vaccine in Gingival Crevicular Fluid

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Summary: GCF is a viable alternative non-invasive source for the detection of antibodies against SARS-CoV-2 S1-RBD antigen following vaccination.

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Abstract

Background: This prospective cohort study aimed to evaluate the antibody responses in non-invasive gingival crevicular fluid (GCF) and unstimulated whole saliva to the SARS-CoV-2 Spike unit 1 receptor-binding domain (S1-RBD) protein following administration of the mRNA BNT162b2 vaccine.

Materials and Methods: This longitudinal study recruited 37 participants with no prior COVID-19 exposure (8 people recruited prior to the COVID-19 pandemic – labelled pre-COVID, 16 vaccinated and 13 non-vaccinated participants). An enzyme-linked immunosorbent assay (ELISA) was used to determine antibody levels against S1-RBD in 90 saliva and 80 GCF samples obtained at 1 and 3 weeks after dose 1, and 3 days, 7 days and 3 weeks after dose 2. To determine previous SARS-CoV-2 infection status, anti-nucleocapsid (N) Ig levels were determined in samples from 8 pre-COVID (saliva as reference), 13 non-vaccinated (saliva and GCF) and 16 vaccinated (saliva and GCF) participants at 1-week post-dose 1 using ELISA.

Results: Salivary levels of Anti-N antibodies measured in samples from vaccinated and non-vaccinated participants were comparable to those in pre-COVID saliva samples collected between October 2018 and September 2019, thus confirming that all study participants had no prior SARS-CoV-2 infection. Overall, the levels of anti-S1-RBD antibodies peaked at 3 weeks after dose 2 in both saliva and GCF for all three immunoglobulin isotypes. Notably, the concentration of anti-S1-RBD antibodies in GCF was significantly higher than in saliva at all time points.

Conclusion: This study establishes GCF and saliva as viable alternative non-invasive sources to monitor levels of antibodies following vaccination, with GCF demonstrating feasibility as a biofluid source for the detection of antibodies against SARS-CoV-2 S1-RBD antigen.

Keywords: COVID, Immunology, Gingival Crevicular Fluid, Biomarker, clinical study, Host response, Periodontics, Saliva
Introduction

SARS-CoV-2 is a newly identified enveloped, RNA single-stranded virus within the Coronaviridae family that is responsible for the coronavirus disease 2019 (COVID-19) pandemic. SARS-CoV-2 has four key structural proteins: the nucleocapsid (N), spike (S), envelope and membrane. Among these proteins, host antibody responses after natural SARS-CoV-2 virus infection are targeted primarily towards the S and N proteins. The spike glycoprotein contains a receptor-binding domain (RBD) that mediates viral binding to the angiotensin-converting enzyme 2 (ACE2) receptor on human cells, and it is a major target for vaccines and rapid antigen tests. Antibody detection assays targeting the RBD protein are useful in seroepidemiological studies, individual risk assessment, and determining the sustainability of the anti-RBD antibody response.

Recent evidence indicates that the oral cavity is an important site for SARS-CoV-2 infection, and that saliva and gingival crevicular fluid (GCF) serve as SARS-CoV-2 virus reservoirs. Saliva is recognised as a powerful non-invasive biosample comprising local and systemic proteins, pathogens and antibodies, and an alternative source to detect immunoglobulin (Ig; IgG, IgM and IgA) antibody responses upon SARS-CoV-2 infection. GCF is another non-invasive highly concentrated biofluid composed of serum and local periodontal tissue constituents that reflects both local and systemic biomarker status. Recently, GCF was shown to share the same kinetics of Ig antibody response with plasma for COVID-19 positive patients, further supporting the potential of GCF as a non-invasive tool for assessing immune status, especially the important issue of waning immunity over time. It is intriguing to explore whether GCF (saliva as control) could be useful non-invasive analytes for assessing the effectiveness of vaccination against SARS-CoV-2 in terms of stimulating immunoglobulin antibody production.

The messenger RNA (mRNA) Pfizer–BioNTech vaccine BNT162b2 developed against SARS-CoV-2 offers great promise to prevent the spread of disease and mitigate morbidity and mortality associated with SARS-CoV-2 infection. This vaccine induces Spike protein-specific immunoglobulin antibodies that confer
protective immunity. IgG, IgM, and IgA antibody responses have been studied extensively in blood (or sera) samples in either COVID-19 patients or BNT162b2 vaccination recipients to gain insights into the host immune response. Since blood sampling is invasive, it is of considerable importance to explore oral biofluids as alternative non-invasive samples for this purpose. Currently, there are two commercial antibody tests to detect antibodies against SARS-CoV-2 after vaccination: 1) neutralizing antibody detection tests (i.e., antibody tittering using a competitive ELISA), and 2) binding antibody tests (i.e., binding with Ig using ELISA). Limited studies to date have shown that IgG antibodies against spike protein generated by BNT162b2 vaccination can be detected in saliva using either binding antibody tests or neutralising antibody test, while the presence of Ig antibodies in GCF following BNT162b2 vaccination has not yet been demonstrated. As both saliva and GCF are reservoirs for SARS-CoV-2 virus and important oral biofluids for Ig detection upon infection, the present study was undertaken to determine SARS-CoV-2-specific antibody levels in GCF and saliva over time, following the first and second dose of the BNT162b2 vaccine.

Materials and Methods

Study participant recruitment

This study was approved by the human subjects ethics board of the Metro North Hospital and Health Service (MNHHS) and The University of Queensland Human Ethics Committees (approval numbers: 65509 and 2020/HE002629, respectively) and was conducted in accordance with the Helsinki Declaration of 1975, as revised in 2013. This study was performed following the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines for human observational investigations. All the participants were voluntary with written informed consent prior to enrolment in the study. All 37 participants (8 pre-COVID, 16 vaccinated and 13 non-vaccinated) were staff and students at the University of Queensland School of Dentistry, being generally healthy without any underlying systemic diseases and with no prior SARS-CoV-2 infection. Consecutive participants who were older than 18 years were included, with no exclusion criteria being applied. Demographic data are presented in Table 1 and in Table S1 in online Journal of Periodontology.
This observational study monitored the levels of antibodies to SARS-CoV-2 antigens in participants who received the BNT162b2 vaccine between May and July 2021 (n=16). Samples of whole unstimulated saliva and GCF were collected at 1 week and 3 weeks after the first dose, and then at 3 days, 7 days, and 3 weeks after the second dose (see Figure S1 in online Journal of Periodontology). There was an interval of 3 weeks between the two vaccine doses and a total follow-up time of 6 weeks. These time points were chosen based on the documented humoral immune response upon viral infection and immune response post-BNT162b2 vaccine 32-34.

IgG, IgA and IgM antibodies to the RBD of the S1 subunit of the viral spike protein (SARS-CoV-2-S1-RBD) were assayed using a commercial enzyme-linked immunosorbent assay (ELISA). To remove any potential bias, samples from participants who were not vaccinated (n=13) and not previously infected by COVID-19 were used as non-vaccinated controls.

To confirm that our participants were not previously COVID-19 positive, we compared the saliva samples to 8 whole saliva samples collected prior to the COVID-19 pandemic. Thus, whole unstimulated saliva from pre-COVID (n=8), non-vaccinated (n=13) and vaccinated (n=16) participants were subjected to ELISA for N protein, which only increases following exposure to COVID-19 but not following vaccination.

It is noted that one participant dropped out after the second dose and two participants did not provide GCF samples. Thus, a total of 98 saliva and 80 GCF was collected for this study at defined time points (see Table S2 in online Journal of Periodontology).

**Saliva and GCF sampling**

All participants were asked to refrain from food and drink for at least 1 hour before their appointment. Samples were collected between 9.00 AM and 12.00 PM. The participants rinsed their mouths to remove any food debris using 10 mL of water before saliva and GCF collection. Saliva was collected first and GCF collection was performed by a registered periodontist within 30 mins after saliva collection.

Unstimulated whole saliva samples were collected using the spitting method as described previously 35-38. The participants expectorated the saliva into a sterile 50ml
falcon tube for 1 min. The samples of whole saliva were kept immediately on ice, and then aliquots were stored at ~80 °C until used for ELISA. After that, 10 µL of whole saliva was carried out for ELISA test.

For consistency, GCF samples were collected from a single non-bleeding mesiobuccal premolar site using sterile strips† by a registered periodontist as described previously 39, 40. The pre-selected mesiobuccal premolar site for each participant did not produce bleeding upon probing. Briefly, the area for collection of GCF was isolated using cotton rolls and air-dried for 10 seconds to avoid saliva contamination. Subsequently, a paper strip was inserted 2-3 mm into the gingival sulcus of the right maxillary premolar tooth (#14) at the mesiobuccal line angle and left for 30 seconds, ensuring that no saliva or blood contamination occurred. The GCF volume (µL) was determined ‡ and ranged between 0.09 and 1.2 µL (see Table S3 in online Journal of Periodontology). After Periotron reading, the paper strips were placed in 1.5mL Eppendorf tubes and eluted with 300 µL of phosphate-buffered saline. The eluate was immediately frozen and kept at −80°C until analysis. 10 µL of diluted GCF eluate was used for subsequent ELISA assay.

**Antibody detection against the SARS-CoV-2 proteins**

Commercially available ELISA assays were used to measure the levels of IgG, IgM and IgA antibodies against the spike glycoprotein § and N protein ** according to the manufacturer’s instructions and as published previously 41. Briefly, 10 µL of saliva and 10 µL of eluted GCF were diluted in sample buffer to 50 µL and added to antigen-coated wells for 1 hour at room temperature. Thus, each saliva sample was diluted 5 times and GCF samples were diluted ~1,100 to 16,000 times for ELISA (see Table S3 in online Journal of Periodontology). After a series of washes with PBS, 50 µL biotinylated anti-human IgG antibody was added to each well, and the samples were incubated for a further 30 minutes. After final washes with PBS, 50 µL of HRP-streptavidin solution was added to each well for 30 minutes at room temperature, followed by 50 µL of TMB One-Step Substrate Reagent and then a

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† PerioPaper, Oraflow Inc., Planviwe, NT, USA
‡ Periotron 8000, Pro-Flow Inc., Amityville, New York, USA
§ catalogue numbers: IEQ-CoVS1RBD-IgG-2, IEQ-CoVS1RBD-IgA-2, IEQ-CoVS1RBD-IgM-2; Raybiotech, Peachtree Corners, GA, USA
** catalogue numbers: IEQ-CoVN-IgG-2, IE-CoVN-IgM-2, IE-CoVN-IgA-2; Raybiotech, Peachtree Corners, GA, USA

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further incubation for 15 minutes at room temperature. The colour reaction was halted with 25 µL of Stop Solution, and the degree of enzymatic conversion of the substrate was determined by measuring absorbance at 450 nm.

As stated in manufacturer’s instructions, the exact amount of IgG/IgM/IgA is not known for positive controls, and as such, raw absorbance values at 450 nm for samples are reported, in line with previous publications \(^{41-43}\). The values of negative controls from the kits were used as internal controls (ODs ranging from 0.067 to 0.073, shown in see Table S4 in online Journal of Periodontology), with cut-off values calculated as the average of 13 negative samples plus the standard deviations (SD), as described in previous reports \(^{11, 12, 20}\). Controls and samples were assayed in duplicates as described previously \(^{10, 11}\). The intraassay variability was evaluated by determining the standard deviation and percent coefficient of variation (CV %); CV % was no greater than 30% for the duplicate values.

A sample was considered IgG/IgM/IgA positive if the absorbance reading value was greater than the cut-off value and internal negative control. For S1-RBD-IgM/IgA, albumin protein background was accounted for by subtracting signals from parallel samples added in duplicate to a second plate of albumin protein-coated wells from sample readings for the S1 RBD plate. As such, instances of ‘negative’ data are presented (Figures 1b and c). Furthermore, protein content of saliva and GCF samples was determined by a BCA Protein Assay \(^{††}\) following the manufacturer’s instructions. Absorbance (OD) was measured at 562 nm on a spectrophotometer \(^{‡‡}\). Immunoglobulin OD values for saliva and diluted GCF were normalised as protein quantity within each 10 µL sample and expressed as OD per ug total protein (Figure 3).

Statistical analyses

Data were analysed and presented as scatter plots displaying median ± 95% confidence interval (CI), unless otherwise defined, using GraphPad Prism \(^{§§}\). Data normality (Gaussian distribution) was determined by the D’Agostino-Pearson test with an \(\alpha\) cut-off at 0.05. Further, the normality test was visualised by quantile-

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\(^{††}\) Pierce BCA Protein Assay Kit, ThermoFisher Scientific, QLD, Australia

\(^{‡‡}\) Tecan Infinite® M200 Pro spectrophotometer, Tecan, Switzerland

\(^{§§}\) v9.0.0, San Diego, CA, USA
quantile plots in Prism, with data points following a straight line that matches the line of identity (if data are normally distributed). Data were considered as Gaussian distribution when they passed both normality tests.

A mixed-effects model (to handle the missing data of dropouts) with Geisser-Greenhouse correction and Dunnett's multiple comparisons test was used to compare S1-RBD Ig levels for each participant at defined time points, relative to 1-week post-dose 1. Wilcoxon matched-pairs signed-rank test (non-parametric data) and paired T-test (parametric data) was applied for the comparison of S1-RBD Ig concentration between saliva and GCF at each time point, with missing data being excluded in this analysis.

Ordinary one-way ANOVA with Tukey's test (parametric data) and Kruskal-Wallis with Dunn's test (non-parametric data) was used to compare N-Ig expression levels between pre-COVID, non-vaccinated and 1-week post-dose 1 vaccinated saliva samples. The GCF N-Ig data were compared using an unpaired T-test (parametric data) and Mann Whitney test (non-parametric data), respectively. A two-tailed p-value < 0.05 was considered statistically significant.

Results

Study subjects

A total of 29 (16 vaccinated, 13 non-vaccinated) subjects were enrolled, who provided saliva and GCF after vaccination with BNT162b2. Among vaccinated people, two participants did not provide GCF samples and one participant withdrew from the study after dose 2. In total, 98 saliva and 80 GCF samples were collected from 16 vaccinated participants, and 13 non-vaccinated participants (see Table S2 in online Journal of Periodontology). The age range of pre-COVID, non-vaccinated and vaccinated participants were comparable being relatively young adults (24 to 51 years old), with both males and females from various ethnic backgrounds (Table 1 and see Table S1 in online Journal of Periodontology). For the 8 pre-COVID participants, 3 males and 5 females were aged 30.5 ± 4.9 years old. In non-vaccinated participants, there were 10 males and 3 females aged 32.2 ± 4.9 years old, while in vaccinated recipients there were 10 males and 6 females at 31.9 ± 9.7 years of age. Pre-COVID, non-vaccinated and vaccinated groups had similar age
and sex profiles, although most participants were male. In terms of post-vaccine symptoms (see Figure S2 in online Journal of Periodontology), injection site pain, fatigue and headache/dizziness/light-headedness were the most commonly reported symptoms following both dose 1 and dose 2. Of note, one participant experienced severe symptoms for more than 7 days after both doses.

The variability (CV%) for the majority of data was smaller than 15% CV% (93.3%). There was no significant difference in salivary anti-N-protein IgG, IgM and IgA between 8 pre-COVID, 13 non-vaccinated and 16 vaccinated participants at 1-week post-dose 1. The same was found in GCF for non-vaccinated and 1-week post-dose 1 vaccinated participants (see Figure S3 in online Journal of Periodontology). This suggests that our participants had no prior SARS-CoV-2 infection at the time of sample collection.

**Antibody responses after BNT162b2 to SARS-CoV-2 S1 protein**

Overall, unadjusted absorbance readings indicated higher median IgG, IgM and IgA levels against SARS-CoV-2-S1-RBD protein in vaccinated recipients compared to non-vaccinated individuals, for both saliva and GCF, although based on small sample size (Figures 1, 2). In terms of temporal patterns, levels of IgG against S1-RBD protein in saliva were not significantly different between doses 1 and 2, while levels of IgG in GCF were significantly higher at all time points (3 weeks after dose 1, and 3 days, 7 days and 3 weeks after dose 2) compared to 1 week after dose 1 (Figure 1a). No significant changes in either salivary or GCF IgM between doses 1 and 2 were observed (Figure 1b), although absorbance readings for IgM were considerably higher in saliva than in GCF. An elevated IgA response was seen in saliva samples, although this was significant only at 3 weeks post dose 2 compared to 1 week after the first dose (Figure 1c). Conversely, there was a significant increase in GCF anti-IgA antibodies at all time points (3 days, 7 days and 3 weeks) post dose 2 (Figure 1c). All three immunoglobulin isotypes reached their peak levels at 3 weeks post dose 2 in both saliva and GCF (Figure 2). In terms of gender, we determined each participant’s antibody response kinetics over time (Figure 3) and the trend between males and females was similar in both saliva and GCF (see Figure S4 in online Journal of Periodontology).
When the percentage of participants exhibiting a positive immune response to S1-RBD protein (i.e. higher than the non-vaccinated cut-off values in Figure 1) was compared at each time point after the first and second doses of vaccine, there was a higher percentage of positive immune responses (IgG/IgM/IgA) to S1-RBD protein in saliva samples than in single-site GCF samples following the first dose (see Table S2 in online Journal of Periodontology). In contrast, the percentage of GCF samples with positive anti-S1-RBD IgG/IgM/IgA responses increased following the second dose, and these were comparable to saliva samples at all time points after vaccination (see Table S2 in online Journal of Periodontology).

Assay samples were normalised to the amount of total protein in the sample volume (OD/µg total protein) for direct comparison between saliva and GCF concentrations of IgG, IgM, and IgA (Figure 4). The concentration of all three Ig isotypes was significantly higher in GCF than saliva at all time points, with an increase in median OD/µg total protein between 2.5- and 16-fold (Figure 4a-c).

Discussion

Monitoring antibody responses after COVID-19 vaccination is critical for understanding the effectiveness of the conferred protection against SARS-CoV-2 infections, and for informing public health measures at the community level. The results of this study show that immunoglobulins against SARS-CoV-2-S1-RBD (IgG/IgM/IgA) generated by the BNT162b2 vaccine are detected in both saliva and GCF. Moreover, GCF was shown to be markedly more sensitive in terms of Ig concentration. This is the first study to demonstrate that the immune response to COVID19 is detectable in GCF and that GCF collected from a single site can be informative for monitoring and assessing immune response after vaccination.

Oral biofluids are valuable as “liquid biopsies” for the detection of antibodies against SARS-CoV-2-S1-RBD. Studies to date have utilised blood, serum, or breast milk to detect antibodies generated by BNT162b2 mRNA vaccination \(^\text{16, 17, 19-25}\). Recently, stimulated saliva \(^\text{27}\) or extracts from saliva filtered through 22 µm filters \(^\text{26}\) were used to track antibodies against the spike glycoprotein. The levels of salivary IgG, IgM and IgA against SARS-CoV-2 S1-RBD protein observed in the current study are consistent with recently published data on antibodies against the spike glycoprotein being present in the saliva of BNT162b2 vaccine recipients \(^\text{26, 27}\). Of note, levels of
antibodies in saliva against S1-RBD peaked 3 weeks after dose 2, which is in line with changes in the levels of antibodies in serum. The levels of IgG antibodies in saliva against the spike protein are approximately 500-times lower than in serum, consistent with the premise that salivary antibodies are a dilution of the concentration found in serum. This may explain why antibody levels in the present study varied between individuals. Indeed, immune response varied among individuals after each vaccination dose (see Table S2 in online Journal of Periodontology). Compared to a blood sample, whether saliva and GCF reflect comparable levels of immunoglobulin response needs further investigation.

Our findings support the value of GCF as an analyte for assessing antibody responses against S1-RBD after vaccination. The evidence for this comes from using matched samples of unstimulated saliva and GCF from the same individual over time. Although raw absorbance readings for IgG, IgM and IgA antibodies against the S1-RBD protein were higher for whole unstimulated saliva than GCF (Figure 1), there was up to a 16-fold increase in concentrations of the Ig isotypes in GCF after normalising for total sample protein (Figure 3). There are other recent data showing GCF to be comparable to saliva in terms of its sensitivity as a diagnostic fluid for the detection of SARS-CoV-2 virus. Despite GCF having the same kinetics of IgA and IgG antibodies upon SARS-CoV-2 infection, it is not feasible to directly compare the antibody concentration in blood and GCF presented in this study, as a direct comparison is not possible with the previously reported blood neutralising antibody titer (>1:160 dilution) and GCF antibody ratio (ng of specific Ig per 100 mg total IgG) data. GCF is a serum transudate, reflecting its constituents and comprising highly concentrated biological components compared to saliva. It is worth noting that in the present study, saliva was diluted 5 times, but GCF was diluted 1,000-16,000 times for ELISA. Due to the limitation of commercial ELISA kits that were used in this study, we were unable to normalise the Ig data to GCF volume, without knowing the exact amount of Ig in positive control samples. In Figure 4, diluted GCF exhibited an Ig increase of 2.5- and 16-fold compared to saliva. Considering the dilution factor of GCF, it is speculated that GCF has at least more than 500 times higher antibodies than saliva, while saliva has ~500 times lower than blood. This suggests that highly concentrated GCF may reflect circulating Ig concentrations following BNT162b2 mRNA vaccination. However, patient-matched
blood samples should be assayed in future studies to confirm this. Moreover, the vaccinated participants belong to the 25-50-year group and our data showed that antibody responses between males and females have a similar trend, which is in line with published immune responses that are gender-independent within the same age group. In light of this, the findings of the present study highlight the potential of GCF as a tool for both COVID-19 diagnosis and immune surveillance available to dentistry practitioners and researchers alike.

It is highlighted that GCF collection needs to be performed by an appropriately trained dental professional; therefore, the application of GCF as a clinical source for immune response monitoring may be limited at this point in time due to the technique sensitivity of collection protocols. Nevertheless, GCF potentially mirrors serum antibody levels and is a non-invasive sampling method more favourable to blood collection. It may be worthwhile to train dental professionals in standardised GCF collection, which could be incorporated into routine dental screening procedures to facilitate non-invasive periodontal biofluids-GCF diagnostics for immune surveillance at a community level.

Several study limitations that must be acknowledged include, i) a relatively small sample size, ii) the absence of baseline (pre-vaccination) data for vaccinated participants, such that it was necessary to use saliva and GCF samples from a separate cohort of non-vaccinated individuals as negative controls, iii) the absence of participant-matched blood or serum samples, iv) a relatively narrow age group (paediatric (<16 y.o) and older than 65-year-old were excluded) and v) no correlation assay was performed: a) between saliva and GCF antibodies level and b) saliva/GCF vs matched plasma samples. This is because children under 16 were not being vaccinated in Australia and the older population (>60y.o.) was prioritized for the Oxford/AstraZeneca vaccine at the time of this study. A larger cohort study where matched pre- and post-vaccination samples of GCF, saliva, and blood collected from different aged participants are necessary to validate the utility of GCF for monitoring an individual’s immune status following vaccination. Future correlation assays to correlate antibody levels between saliva and GCF and plasma will be helpful to validate our ‘proof-of-concept’ data and confirm the value of GCF as an alternative non-invasive biosample that reflects systemic antibody status. Notwithstanding the limitations of this study, it is the first report to describe the
utilisation of GCF to detect immunoglobulin antibodies following administration of the BNT162b2 vaccines.

Conclusion

The current data are significant in that they show that the oral biofluids saliva and GCF both represent potential sources for monitoring an individual’s immune status after vaccination, with the more sensitive single-site GCF monitoring being an approach that is non-invasive and well suited to applications in the general clinical dental setting.

Author Contributions

P. Han and S. Ivanovski contributed to funding acquisition, conception, participant recruitment, clinical sample collection, design, data interpretation, drafted and critically revising the manuscript. C.S. Moran, S.S. Ramachandra and L.J. Walsh contributed to participant recruitment, data acquisition, drafted and critically revised the manuscript.

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Declaration of Conflicting Interests

The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article. All authors gave their final approval and agree to be accountable for all aspects of the work.
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Figure legends

Figure 1. Longitudinal analysis of IgG (a), IgM (b) and IgA (c) response to the spike 1- RBD antigens of SARS-CoV2 in saliva and GCF. ELISA absorbance values are shown as scatter plots, with medians and 95% confidence intervals. The blue dotted line represents cut-off values from non-vaccinated individuals. *p<0.05, **p<0.002, ****p<0.0001 vs matched individuals at 1-week after dose 1

Figure 2. Immunoglobulin response to S1-RBD over time in saliva and GCF (based on raw readings in Figure 1). Data are presented as median ± 95% CI.

Figure 3. Patient matched samples are represented by the connecting lines. Blue and red lines represent samples from male and female participants, respectively.

Figure 4. Higher levels of IgG (a), IgM (b) and IgA (c) antibodies response in GCF compared to saliva following two doses of Pfizer-BioNTech vaccine. Data were calculated using raw absorbance values per ug total protein. Data are presented as median ± 95% CI. **p<0.002, ***p<0.001, ****p<0.0001.
Figure 1. Longitudinal analysis of IgG (a), IgM (b) and IgA (c) response to the spike 1-RBD antigens of SARS-CoV2 in saliva and GCF. ELISA absorbance values are shown as scatter plots, with medians and 95% confidence intervals. The dotted line represents cut-off values from non-vaccinated individuals. * p < 0.05, † p<0.002, ‡ P<0.0001 between groups compared to vs matched individuals at 1-week after dose 1.
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| Variables | Gender/ Ethnicity | Pre-COVID | Non-vaccinated | Post-vaccine dose 1 | Post-vaccine dose 2 |
|-----------|----------------|-----------|----------------|---------------------|---------------------|
| n         |                | 8         | 13             | 16                  | 15                  |
| Age in years (mean ± SD) |                | 30.5±4.9  | 32.2 ± 4.9 (24-38) | 31.9 ± 9.7 (25-43) | 31.4 ± 4.1 (25-43) |
| *P*       |                | >0.9      | 0.56           | 0.48                |
| (for age) |                |           |                |                     |
| Gender, n (%) | Male    | 3 (37.5%) | 10 (76.9%) (62.5%) | 10 (62.5%)         | 9 (60%)            |
| Ethnicity, n (%) | Female | 5 (62.5%) | 3 (23.1%) (37.5%) | 6 (37.5%)          | 6 (40%)            |
| Caucasian |                | 2 (25%)   | 5 (38.5%) (18.8%) | 3 (18.8%)          | 3 (20%)            |
| Asian     |                | 6 (75%)   | 7 (53.8%) (68.7%) | 11 (73.3%)         |                    |
| Other     |                | 1 (7.7%)  | 2 (12.5%)       | 1 (6.7%)           |                    |
**Note:** Pre-COVID: participants that were recruited before the COVID-19 pandemic between October 2018 and September 2019.

* p values were calculated using the Kruskal-Wallis test and Dunn's multiple comparisons test vs non-vaccinated participants.