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Short Communication

Immunoglobulin A response to SARS-CoV-2-N-protein potentially persists in oral fluids of patients with periodontitis six months after mRNA vaccine administration

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Abstract Few studies have investigated the mucosal immune response after BNT162b2-booster vaccination in individuals with periodontitis. In this study, we evaluated the persistence of IgA anti-SARS-CoV-2-N-protein in saliva and gingival crevicular fluid (GCF) of patients with periodontitis for at least six months post BNT162b2 vaccine booster. We included patients with moderate (n = 7) and severe (n = 7) periodontitis and participants without periodontitis (n = 7) as controls. The Bradford method measured the protein concentrations in the samples, and an enzyme-linked immunosorbent assay of the SARS-CoV-2 N protein was performed to analyze the targeted IgA level. For the tested SARS-CoV-2 antigen (N-protein), IgA levels in saliva and GCF showed a strong and significant correlation. Therefore, in patients with moderate or severe periodontitis, saliva and GCF can provide information regarding the IgA response against SARS-CoV-2-N-protein. The neutralizing activity of IgA against SARS-CoV-2 was not investigated in this study, necessitating further research.

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Introduction

Previous studies indicate that periodontitis is a risk factor associated with adverse COVID-19-associated outcome.[1] However, to date, there are limited studies evaluating the mucosal immune response in patients with periodontitis after the administration of either primary or booster doses of SARS-CoV-2 vaccination. Additionally, as in other countries, vaccination programs in Indonesia are recommended for all individuals, including those with oral diseases such as periodontal disease. To date, no studies have been conducted on the immunogenicity of booster-dose mRNA vaccines among Indonesian patients with periodontitis.

As shown in the literature, messenger RNA (mRNA), such as Pfizer-BioNTech COVID-19 and Moderna COVID-19 vaccines, stimulate an immune response. This response can prevent the spread and mitigate the morbidity and mortality associated with SARS-CoV-2 infection.[2] Therefore, this study aimed to evaluate the immunoglobulin (IgA) levels in the saliva and GCF of participants with and without periodontitis, six months after administration of two intramuscular COVID-19 mRNA vaccine doses. IgA was chosen for evaluation because it plays an important role in mucosal immunity and viral neutralization.[3] Saliva and GCF were selected as samples because they can provide valuable information regarding the IgA response against SARS-CoV-2 infection.[4]

Materials and methods

Participants recruitment

This observational study was conducted in May 2023 following the acquisition of consent from the participants (ethics approval ref. 90/Ethical Approval/FKG UI/X/2022) before saliva collection. This study was performed according to the guidelines of the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement.[5] All clinical data and information were obtained from hospital reports (data not shown). The target participants for this study were individuals who had received the BNT162b2 vaccine (either the Pfizer-BioNTech COVID-19 or the Moderna COVID-19 vaccine) at least six months before the study. Participants aged >18 years with no prior SARS-CoV-2 infection were included in this study. Except for patients aged (>60 years), no exclusion criteria were applied. All recruited participants received their first and second doses of the COVID-19 vaccine (Sinovac-CoronaVac vaccine), as confirmed through the application (PeduliLindungi app) provided by the Indonesian government.

Of the 21 participants, 14 had moderate (G1; n = 7) or severe (G2; n = 7) periodontitis, whereas participants without periodontitis served as controls (n = 7). The diagnosis of periodontitis (moderate to severe) was determined according to established criteria, as reported in a previous study.[5]

Saliva and gingival crevicular fluid sampling

Participants were asked to abstain from food and drinks (at least 1 h) prior to the collection of saliva and GCF samples between 8:00 a.m. and 11:00 a.m. Whole, unstimulated saliva samples were collected by spitting into Falcon tubes, whereas GCF samples were collected from a single non-bleeding premolar tooth (mesiobuccal site) using a paper point. The obtained collected oral samples were placed immediately on ice and stored at −80 °C until further use for enzyme-linked immunosorbent assays (ELISA).[6] Furthermore, the total protein concentration in saliva and GCF was measured using the Bradford protein assay, while the level of IgA reactive to the nucleocapsid protein of SARS-CoV-2 was quantified using an ELISA kit (ImmunoDiagnostic Limited, Sha Tin, Hong Kong) according to the manufacturer's instructions. A sample was considered positive for IgA when the optical density (OD) was ≥0.1, while concentrations were interpreted as negative when the OD was <0.1.

Statistical analysis

Descriptive data were presented as median (range), and analyses were performed using GraphPad Prism software (San Diego, CA, United States). One-way ANOVA with Kruskal–Wallis (non-parametric data) was used to compare the total protein concentration and salivary IgA levels among the groups (Control, G1, and G2). Statistical comparisons between the periodontitis groups (G1 and G2) were performed using an unpaired Student's t-test. Two-tailed *P*-values <0.05, were considered significant. For correlation analysis, Spearman's correlation coefficient (r) with two-tailed *P*-values was calculated and linear regression was applied to generate the line of best fit with 95% confidence intervals.

Results

Of the 21 participants recruited, 14 were diagnosed with periodontitis and 7 were not. The patients with periodontitis were divided into moderate (G1, n = 7) and severe (G2, n = 7) groups. In both groups, the age range of participants was comparable, with all participants being relatively young adults (25–45 years).

First, the total protein concentrations in saliva and GCF were compared between the groups. Fig. 1A shows the measured concentrations of salivary and GCF proteins in all participants (100%). There was no significant difference in the total salivary protein concentration between the control and periodontitis groups (G1 and G2). However, in the GCF, the total protein levels in the G1 and G2 groups were significantly higher than those in the control group (Fig. 1B; $P = 0.001$).

The unadjusted absorbance reading indicated that the median IgA levels against SARS-CoV-2-N-protein were detected in all (100%) saliva samples (S-IgA). However, GCF-IgA was only detected in six (85.1%) and five (71.4%) participants in G1 and G2, respectively. In the control group, this antibody was detected in one participant (14.2%). Moreover, there was a higher median S-IgA level against the SARS-CoV-2-N-protein in vaccinated individuals among patients with periodontitis than in individuals in the control group (Fig. 1C, $P = 0.003$). However, in the G1 and G2 groups, S-IgA titers were not significantly

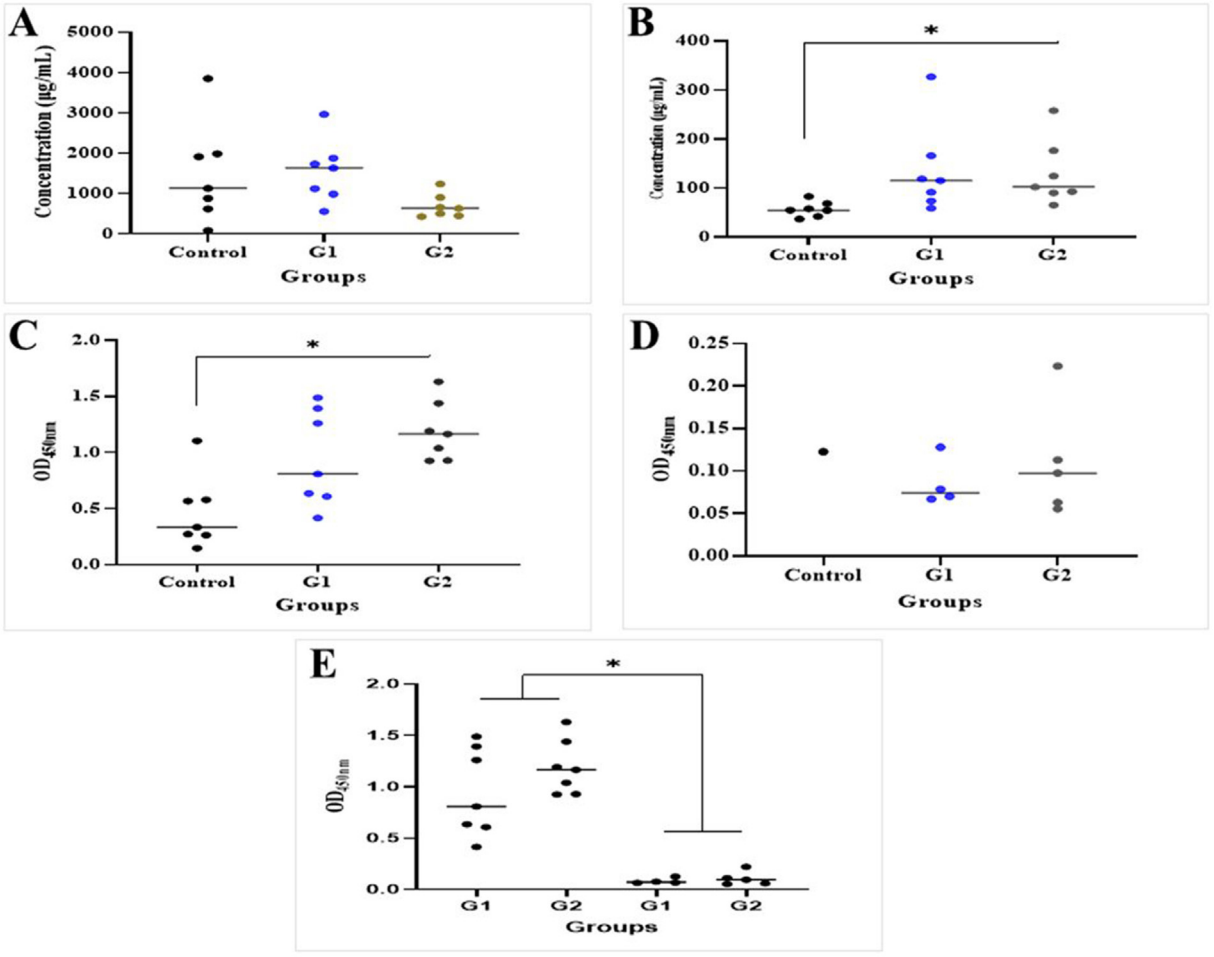

Figure 1 Evaluation of protein concentrations and the level of IgA anti-SARS-CoV-2-N-protein in saliva and gingival crevicular fluid of periodontitis (moderate [G1] and severe [G2]) and non-periodontitis groups (control subjects). Generally, the different concentrations of salivary protein between the three groups are not significant (A). The concentration of GCF-protein in periodontitis groups is significantly higher ($P = 0.001$) compared to those observed in the control group (B). The levels of S-IgA in periodontitis groups were found to be higher than the levels of the control group ($P = 0.003$) (C), while the different levels of GCF-IgA were not significant (D). The level of S-IgA was significantly higher compared with the levels of GCF-IgA ($P = 0.01$) in the periodontitis groups (E). GCF = gingival crevicular fluid. S-IgA = secretory IgA.

different. Similarly, the levels of GCF-IgA in G1 and G2 were not significantly different (Fig. 1D). Interestingly, in the periodontitis group, the salivary IgA levels were significantly higher than the level of GCF-IgA (Fig. 1E, $P = 0.001$).

The correlation between the amount of IgA in the saliva and GCF was further analyzed. As shown in Fig. 2A and B, a strong and significant association was observed in G1 ($r = 0.88$, $P = 0.03$) and G2 ($r = 0.86$, $P = 0.01$), respectively.

Discussion

This study focused on patients with moderate and severe periodontitis because COVID complications were higher in these patients than in healthy individuals or in patients with mild periodontitis.[7] Our data showed that the concentrations of total salivary proteins in the three groups were statistically similar. In contrast, the protein levels in the GCF of healthy sulci (control group) was lower than that in the periodontitis group. Additionally, the protein concentrations in the G1 and G2 groups did not differ. This result indicates that periodontal inflammation increased GCF flow. Particularly, GCF-IgA was only detected in one person with a healthy sulcus. Both the G1 and G2, in contrast, groups exhibited significantly higher levels of S-IgA and GCF-IgA than that in the control group following booster dose vaccination.

Furthermore, we investigated the association between S-IgA and GCF-IgA levels and COVID-19 in patients with periodontitis. We observed a strong positive linear correlation between the values of S-IgA and GCF-IgA against the SARS-CoV-2-N-protein in groups G1 and G2. It is possible that the increased levels of S-IgA and GCF-IgA after booster vaccine administration were excluded from the serum.[8] Our participants were immunized with the Sinovac-CoronaVac vaccine. Hence, it is reasonable to assume that the

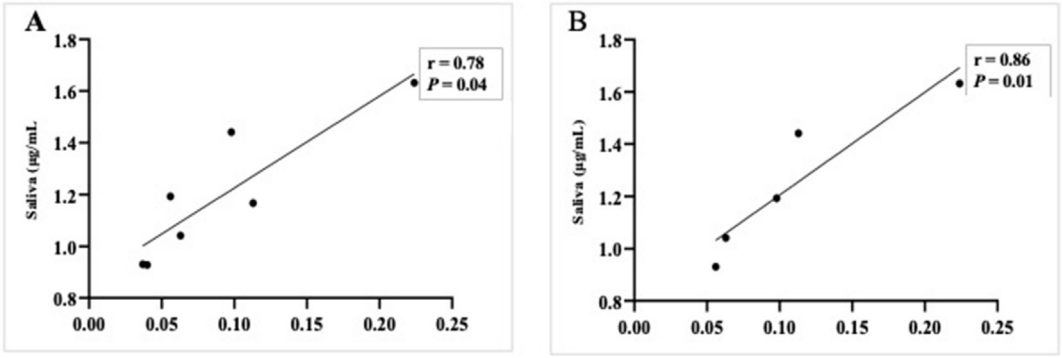

Figure 2 Scatter diagram illustrating the correlation between S-IgA and GCF-IgA in participants with moderate (A) and severe (B) periodontitis. These observations indicate that in periodontitis groups, both the levels of S-IgA and GCF-IgA show a strong significant positive correlation ($P = 0.001$). Spearman correlation coefficient (r^2) and exact *P*-value are provided. GCF-IgA = gingival crevicular fluid-immunoglobulin A. S-IgA = Secretory IgA.

BNT162b2 vaccine, when administered intramuscularly as a booster, may provide long-lasting mucosal immunity. This may reduce or minimize the risk of SAR-CoV-2 infection.

Given that GCF-IgA appears to correlate with systemic antibody levels against SARS-CoV-2[9] and the simplicity of saliva collection,[5] we suggest that detecting N-antigens in SARS-CoV-2 antibody testing using oral fluids may serve as an early tool to evaluate population immunity and vaccine response, particularly in vulnerable populations (as those with periodontitis) or when blood collection is not feasible.

In conclusion, while SARS-CoV-2 spike-specific serum IgA levels decline quickly after natural infection,[10] the results of this study suggest that IgA defenses against SARS-CoV-2-N-protein can persist in oral fluids for six months at least. However, this study has limitations. First, it is unclear whether IgA observed in oral samples represents a protective antibody against SARS-CoV-2 exposure. Second, we were unable to ascertain whether the targeted IgA included the dimeric isoform, which has a more potent neutralizing capacity than monomeric IgA.[10] Lastly, the study was conducted with a relatively small number of participants. Considering the limitations of this study, the severity of periodontitis may not affect salivary IgA levels against the SARS-CoV-2-N-protein.

Declaration of competing interest

The authors have no conflicts of interest relevant to this article.

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