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ORIGINAL ARTICLE

Utilization of Saliva for Anti-SARS-CoV-2 Antibody Detection in the General Population

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ABSTRACT

Background: **Similar to how salivary cortisol reflects serum cortisol as a stress biomarker, saliva by its easy, non-invasive collection, holds promise for mucosal antibody testing to assess systemic humoral immunity. In this study, we aimed to determine the prevalence of SARS-CoV-2 antibodies in saliva during the COVID-19 pandemic.**
Methods: **Between February and December 2022, paired saliva and serum specimens were collected twice per participant with the history of infection. SARS-CoV-2 antibodies were measured by Roche Elecsys anti-N and Abbott anti-S1-RBD assays. Cortisol was also quantified in serum-saliva paired samples using Elecsys Cortisol II. Saliva antibody results with manufacturer's serum cutoff were compared with those of the serum.**
Results: **Out of the 166 participants enrolled, the first and second visits showed serum anti-S1-RBD Ab in 162 (97.6%) and anti-N Ab in 79 (47.5%) and 97 (58.4%) participants. At the first and second visits, 14 (8.4%) and 13 (7.8%) participants had salivary anti-S1-RBD Ab, while 4 (2.4%) and 5 (3.0%) participants had anti-N Ab. Agreement between serum and saliva was slight for anti-S1-RBD ($\kappa = 0.004$) and poor for anti-N ($\kappa < 0$). The mean serum cortisol was 7.52 µg/dL (visit 1) and 7.97 µg/dL (visit 2) versus 0.19 µg/dL and 0.18 µg/dL in saliva. Saliva cortisol levels were ~ 95.5 - 97.6% lower than serum.**
Conclusions: **Salivary SARS-CoV-2 antibody levels and serologic results are poorly concordant, preventing saliva as a direct substitute without assay-specific cutoffs. Salivary cortisol correlates predictably with serum levels but consistently underestimates them. Further work is needed to establish standardized saliva thresholds for both antibodies and cortisol.**
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KEYWORDS

COVID-19 pandemic, saliva, SARS-CoV-2 antibody, non-invasive biomarker

INTRODUCTION

In 2022, the Republic of Korea recorded 28,424,635 COVID-19 cases [1]. Notably, Korea has achieved one of the highest vaccination rates worldwide, with 87.1% of the population fully vaccinated, exceeding the global average booster dose by over twofold as of November

18, 2022 [2]. It is important to know the current status of salivary antibody titers in the vaccinated population. Saliva presents several benefits as a diagnostic medium owing to its non-invasive nature, comfort, safety, and convenience [3]. Evidence supports the efficacy of saliva as a medium for detecting antibodies against diverse viral agents, with a significant association demonstrated between salivary and serum antibodies in the testing of human immunodeficiency virus antibodies and hepatitis A, B, and C, and rubella [4,5].

Pre-existing antibodies in oral fluid and nasopharyngeal lavage, acquired through vaccination or prior infection, play a critical role in preventing reinfection with emerging variants of SARS-CoV-2, such as Omicron [6,7]. Theoretically, an identical population of antibodies can envelop infectious virions, potentially restricting their transmission when encapsulated in aerosols [8]. In contrast to immunoglobulins detected in serum or plasma, threshold values of spike and nucleocapsid-specific IgG and IgA in mucosal sites may serve as more accessible and specific clinical indicators of immunity. This insight could impact decisions regarding the necessity of booster vaccinations in vulnerable and high-risk populations [9].

Given the presence of viral RNA in saliva, the mucosae and draining lymph nodes of the oral and nasopharyngeal tracts are postulated to function as an initiation site for the immune response against SARS-CoV-2 [10]. Recent investigations into antibodies against SARS-CoV-2 have utilized saliva specimens obtained by using Salivette or comparable containers [11-14], as well as aspiration or splitting techniques, employing enzyme-linked immunosorbent assay (ELISA), multiplex bead assay, electrochemiluminescence immunoassay (ECLIA), flow cytometry, and chemiluminescence immunoassay (CLIA) for detecting salivary antibodies.

In this study, we aimed to determine the prevalence of SARS-COV-2 antibodies in the saliva of healthy individuals during the COVID-19 pandemic using two commercial automated immunoassays. Furthermore, we sought to establish a correlation between the presence of antibodies in both saliva and serum.

MATERIALS AND METHODS

Study population

A total of 166 participants were enrolled in this study between February and December 2022. Residual serum and saliva specimens, obtained during the collection and pre-treatment of samples for two clinical performance trials on health function foods and provided secondarily with informed consent, served as resources for our study. The study protocol was reviewed and approved by the Institutional Review Board of PNUYH (approval number: 05-2022-029). Before study participation, 28 and 133 participants had received two and three doses of the SARS-COV-2 vaccination, respectively. Additionally, 61 participants reported a history of prior COVID-19 infection before their inclusion in the clinical trials. However, the information on COVID-19 infection could not be acquired during the period between the first and second visits.

Sample preparation

Participants underwent two hospital visits, with an 8-week interval between each visit (first and second). Paired serum and saliva specimens were collected twice during the study period. Saliva specimens were collected using a Salivette® Cortisol device (Sarstedt AG & Co. KG, Germany) following the manufacturer's instructions. Venous blood samples were collected from participants in serum separation tubes (Greiner Bio-One, Kremsmünster, Austria) and centrifuged at 1,912 × g for 7 minutes. Acquired serum and saliva samples were dispensed into 1.5 mL polypropylene microcentrifuge tubes (Corning Axygen, Corning, NY, USA) within 2 hours and stored at - 70 °C until analysis.

SARS-CoV-2 antibody test

SARS-CoV-2 antibody tests were performed using automated serological immunoassays. Nucleocapsid protein-based Elecsys anti-SARS-CoV-2 assay (anti-N Ab, Roche Diagnostics, Rotkreuz, Switzerland) was performed using Cobas e801 (Roche diagnostics, Mannheim, Germany), following the manufacturer's instructtions, with a cutoff value of 1.0 cutoff index (COI). Additionally, the spike protein-targeted Abbott SARS-CoV-2 IgG II Quant assay (anti-S1-RBD Ab, Abbott, Chicago, IL, USA) was performed on the Alinity i (Abbott Laboratories, Wiesbaden, Germany), according to the manufacturer's instructions, with a cutoff of 50 AU/mL. Since the retesting with dilution was not performed, a value of ≥ 40,000 was considered 40,000.

Cortisol analysis test

A total of 134 volunteer subjects were enrolled in this clinical study. Both saliva and serum specimens were obtained during morning hours (9:00 AM to 11:00 AM) at each visit. Quantitative measurement of cortisol in both serum and saliva specimens was performed using the Roche cobas® e 801 analyzer. The Elecsys® Cortisol II reagent, which is specifically designed for the cobas® e 801 analyzer, was employed for precise quantification of cortisol concentrations in serum and plasma samples. This reagent operates based on electrochemiluminescence immunoassay (ECLIA) technology. All samples were processed according to standardized laboratory protocols to ensure consistency in analytical conditions.

Statistical analyses

Statistical analyses were performed using MedCalc statistical software version 20.218 (MedCalc Software Ltd.). Qualitative data were analyzed using Cohen κ and Pearson χ^2 tests, whereas quantitative data were analyzed using Pearson's correlation coefficient. The significance of group parameter differences was evaluated

using paired and independent *t*-tests, with statistical significance set at $p < 0.05$. Interpretation of κ values followed the following criteria: almost perfect agreement (0.81 - 1.0), substantial agreement (0.61 - 0.8), moderate agreement (0.41 - 0.6), fair agreement (0.21 - 0.4), slight agreement (0.0 - 0.2), poor agreement (below 0) [15].

The relationship between salivary and serum cortisol concentrations was assessed using two complementary statistical approaches. Passing-Bablok regression analysis was performed to evaluate the linear relationship between cortisol measurements in the two sample types. Bland-Altman analysis was conducted to assess the agreement between the two measurement methods and quantify the difference in cortisol concentrations between serum and saliva samples.

RESULTS

Positive rate of tests and distribution of SARS-Cov-2 antibody levels based on SARS-CoV-2 infection and vaccination history

The positivity rates and agreement of antibody testing results during the first and second visits are presented in Table 1. Serum exhibited positive rates ranging from 58.4% to 98.2%, whereas saliva exhibited rates ranging from 2.4% to 8.4%. Among the 161 participants who received SARS-COV-2 vaccination, 160 (99.4%) were consistently positive for anti-S1-RBD Ab during both visits, whereas 87 and 69 participants were positive for anti-N Ab during the first and second visits, respectively.

Anti-S1-RBD Ab levels in saliva were detected in the range of 0 - 328.5 AU/mL, amounting to approximately 0.82% of the upper detection limit observed in serum (0 to > 40,000 AU/mL). Anti-N Ab levels ranged from 0.1 to 2.2 COI in saliva and from 0.1 to 174.0 COI in serum. Serum levels of anti-N Ab, anti-S1-RBD Ab, and salivary anti-S1-RBD Ab levels were higher in participants with a history of SARS-CoV-2 infection than in those without infection history among vaccinated participants (Table 2).

Agreement of the same test across collection times

Perfect agreement was observed over time for serum samples ($\kappa = 0.764 - 0.854$), whereas poor agreement was observed for saliva samples ($\kappa = 0.200 - 0.314$). The correlation coefficient of anti-S1-RBD Ab and anti-N Ab quantitative results between the first and second visits varied ($r = 0.415 - 0.803$) (Figure 1). The second visit yielded a higher concentration of serum anti-N Ab; the means of the first and second visits were 11.5 (95% confidence interval [CI], 8.4 - 14.6) vs. 17.4 (95% CI, 13.0 - 21.8) COI, respectively. However, no discernible difference in quantitative results was observed for the other antibodies.

Agreement between anti-S1-RBD Ab and anti-N Ab tests in specimens collected at the same time

The agreement between anti-S1-RBD Ab and anti-N Ab for both serum and saliva specimens was poor (κ, -0.029 and -0.042, respectively). The antibody level correlation between anti-S1-RBD Ab and anti-N Ab titers collected at the same time is shown in Figure 2. No significant relationship was observed between saliva anti-S1-RBD Ab and anti-N Ab levels ($r = -0.016$, $p = 0.761$), whereas a weak positive relationship was observed between serum anti-S1-RBD Ab and anti-N Ab levels ($r = 0.298$, $p < 0.001$).

Agreement of the same tests across specimens collected at the same time

Agreement of anti-S1-RBD Ab levels between saliva and serum was slight ($\kappa = 0.004$), whereas it was poor for anti-N Ab ($\kappa = -0.043$). The antibody level correlation between saliva and serum samples collected at the same time is shown in Figure 3. A moderately positive relationship was observed between serum and saliva anti-S1-RBD Ab levels ($r = 0.543$, $p < 0.001$); however, no significant relationship was observed between serum and saliva anti-N Ab levels ($r = 0.049$, $p = 0.368$). Notably, in cases of strong anti-S1-RBD Ab levels in the serum, antibodies were detected in some saliva samples; however, the levels proved inadequate for positive interpretation.

Positive anti-N or anti-S1-RBD Ab in saliva samples

The test results for participants with positive salivary antibodies are summarized in Table 3. In at least one of their two visits, 8 participants tested positive for saliva anti-N Ab (1.1 - 1.9 COI), whereas 22 tested positive for saliva anti-S1-RBD Ab (50.4 - 328.5 AU/mL). Only six participants, one with anti-N Ab and five with anti-S1-RBD Ab, had positive results at both time points. There were none in which both salivary antibodies were positive.

Seronegative group

All anti-N Ab seronegative participants (n = 68) exhibited serum anti-S1-RBD Ab, whereas 3 and 7 participants exhibited salivary anti-S1-RBD Ab and anti-N Ab, respectively. All participants in the anti-S1-RBD Ab seronegative group (n = 4) exhibited serum anti-N Ab; however, neither anti-S1-RBD Ab nor anti-N Ab were detected in their saliva.

Comparison of serum and saliva cortisol concentrations

Analysis of cortisol concentrations revealed substantial differences between serum and saliva measurements in Table 4. Serum samples demonstrated markedly higher cortisol concentrations compared to saliva samples. The Serum_1 group at 7.519 μg/dL (95% CI: 6.910 - 8.129) and the Serum_2 group at 7.968 μg/dL (95% CI: 7.353 - 8.583) showed the highest mean cortisol concentrations. In contrast, saliva samples exhibited significantly lower

Table 1. Positive rates and agreement of antibody tests (n, %).

		1st visit	2nd visit	Agreement between 1st and 2nd visit (%)	Kappa (95% CI)
Serum	Anti-S1-RBD Ab	163 (98.2%)	162 (97.6%)	99.4	0.854 (0.572 - 1.000)
	anti-N Ab	79 (47.5%)	97 (58.4%)	88.0	0.761 (0.665 - 0.856)
Saliva	Anti-S1-RBD Ab	14 (8.4%)	13 (7.8%)	89.8	0.314 (0.069 - 0.560)
	anti-N Ab	4 (2.4%)	5 (3.0%)	95.8	0.200 (-0.167 - 0568)

CI: confidence interval.

Table 2. SARS-Cov-2 antibody levels at first visit based on SARS-CoV-2 infection history among vaccinated participants (n = 149).

Mean (95% CIs)	No infection history (n = 90)	Infection history (n = 59)	p-value
Saliva anti-N Ab (COI)	0.2 (0.2 to 0.3)	0.2 (0.1 to 0.2)	0.2348
Saliva anti-S1-RBD Ab (AU/mL)	10.3 (6.1 to 14.4)	31.0 (16.6 to 45.5)	0.0014
Serum anti-N Ab (COI)	3.5 (0.5 to 6.5)	20.5 (15.4 to 25.6)	< 0.0001
Serum anti-S1-RBD Ab (AU/mL)	7,932 (5,907.6 to 9,958.1)	20,791.0 (17,336.1 to 24,245.9)	< 0.0001

All 149 participants were included (five individuals who were not vaccinated and 12 individuals with unconfirmed infection history were not included).
CI: confidence interval.

cortisol concentrations, with Saliva_1 at 0.191 µg/dL (95% CI: 0.162 - 0.220) and Saliva_2 at 0.180 µg/dL (95% CI: 0.158 - 0.203). Median values followed a similar pattern, confirming the substantially lower cortisol levels in saliva compared to serum. Variability in cortisol measurements differed considerably between sample types. Serum samples displayed greater variability, as evidenced by higher standard deviations (Serum_1: 3.5666; Serum_2: 3.599) compared to saliva samples (Saliva_1: 0.1682; Saliva_2: 0.1315).

Regression analysis between saliva and serum cortisol

The relationship between salivary and serum cortisol concentrations was analyzed using Passing-Bablok regression, a robust non-parametric method that is resistant to the influence of outliers and does not require specific assumptions about the distribution of errors. During the first visit, the results of the Passing-Bablok regression analysis demonstrated a proportional increase in salivary cortisol levels at approximately 3.26% of serum concentrations, indicating a positive linear relationship (regression equation: Saliva_1 = -0.0064 + 0.0326 × Serum_1). The scatter plot (Figure 4) illustrates this relationship, with the regression line (in red) demonstrating that salivary cortisol concentrations increase proportionally with serum cortisol levels, albeit at a substantially lower magnitude. Analysis of the second visit data yielded the following Passing-Bablok regression equation: Saliva_2 = -0.0129 + 0.0234 × Serum_2. As shown in Figure 4, the slope coefficient (0.0234) was lower than that observed during the first visit (0.0326), suggesting a reduced rate of change in salivary cortisol relative to serum cortisol during the second assessment. In both visits, salivary cortisol concentrations were consistently lower than their corresponding serum values, with salivary levels representing approximately 2.3 - 3.3% of serum concentrations. The y-intercept values were close to zero in both regression equations (-0.0064 and -0.0129 for the first and second visits, respectively), indicating minimal systematic bias between the two measurement methods at very low cortisol concentrations.

Relative differences between saliva and serum cortisol

Bland-Altman plots were generated using relative differences [(serum-saliva)/serum × 100] against serum cortisol values to assess agreement between serum and saliva cortisol measurements. For the first visit, the mean relative difference was 96.52% (Figure 5.), indicating that on average, saliva cortisol was 95.52% lower than serum cortisol. The limits of agreement (± 1.96 × SD) showed considerable variability, suggesting poten-

Table 3. All cases with positive anti-N or anti-S1-RBD Ab in salivary samples.

	Salivary anti-N Ab (COI)		Salivary anti-S1-RBD Ab (AU/mL)		Serum anti-N Ab (COI)		Serum anti-S1-RBD Ab (AU/mL))	
Case number	1st visit	2nd visit	1st visit	2nd visit	1st visit	2nd visit	1st visit	2nd visit
1	**1.10**	0.83	37.50	41.20	**97.60**	**136.00**	**19,095.8**	**11,604.8**
2	**1.34**	0.11	23.60	6.70	0.08	0.07	**18,289.4**	**9,837.0**
3	**1.10**	0.83	8.40	0.00	0.07	0.07	**4,493.8**	**3,120.3**
4	0.35	**1.13**	0.80	4.60	0.08	0.08	**1,496.0**	*941.4*
5	0.35	**1.77**	7.30	6.90	0.08	0.07	**6,307.9**	*3,462.2*
6	0.73	**1.85**	0.00	0.20	0.19	0.19	**473.9**	**1,251.5**
7	0.64	**1.86**	3.80	1.30	0.07	0.08	**2,143.5**	**969.1**
8	**1.38**	**1.56**	0.00	0.00	0.06	0.06	**370.7**	**338.1**
9	0.19	0.09	**50.70**	19.20	**14.00**	**9.99**	**≥ 40,000**	**27,775.3**
10	0.19	0.33	**60.90**	25.20	**15.70**	**24.60**	**16,037.8**	**7,497.3**
11	0.11	0.13	**61.40**	18.80	**17.10**	**39.90**	**20,754.0**	**11,771.8**
12	0.20	0.15	**63.30**	44.10	**15.00**	**22.00**	**21,621.8**	**16,069.7**
13	0.15	0.16	**70.00**	43.40	**35.30**	**24.60**	**27,032.4**	**10,550.2**
14	0.21	0.17	**77.90**	32.50	**3.90**	**2.16**	**≥ 40,000**	**29,088.3**
15	0.17	0.14	**92.00**	42.70	0.08	0.08	**≥ 40,000**	**25,131.1**
16	0.13	0.12	**94.90**	8.20	0.08	0.08	**5,246.0**	**3,924.4**
17	0.46	0.15	**102.30**	4.10	**39.70**	**26.70**	**≥ 40,000**	**≥ 40,000**
18	0.17	0.18	35.30	**51.20**	**25.20**	**17.60**	**24,631.4**	**19,407.2**
19	0.13	0.10	15.30	**52.50**	**3.36**	**14.30**	**30,732.4**	**≥ 40,000**
20	0.12	0.08	45.40	**56.30**	0.45	**52.90**	**33,066.3**	**≥ 40,000**
21	0.24	0.18	12.60	**59.00**	0.16	0.16	**5,239.7**	**17,025.5**
22	0.11	0.11	3.90	**74.90**	0.08	**29.10**	**8,433.6**	**31,186.8**
23	0.19	0.34	28.60	**93.90**	**3.88**	**59.00**	**8,459.2**	**17,693.5**
24	0.13	0.16	0.00	**124.50**	0.08	**50.70**	**2,635.9**	**27,584.4**
25	0.64	0.26	46.80	**166.80**	**30.50**	**22.60**	**2,2333.2**	**19,415.6**
26	0.16	0.20	**50.40**	**110.50**	**7.93**	**5.28**	**≥ 40,000**	**≥ 40,000**
27	0.35	0.58	**57.90**	**99.30**	0.97	**11.60**	**34,879.3**	**24,219.8**
28	0.27	0.41	**85.20**	**92.20**	**10.10**	**5.55**	**≥ 40,000**	**36,539.7**
29	0.17	0.15	**274.60**	**242.60**	**43.80**	**78.60**	**≥ 40,000**	**≥ 40,000**
30	0.15	0.15	**328.50**	**187.20**	**29.60**	**35.50**	**≥ 40,000**	**≥ 40,000**

Positive results are underlined.

Table 4. Quantitative analysis of cortisol in the patients' serum and saliva.

Group	n	Mean (µg/dL)	Median (µg/dL)	SD	95% CI (µg/dL)
Saliva_1	134	0.191	0.144	0.1682	0.162 to 0.220
Saliva_2	134	0.18	0.15	0.1315	0.158 to 0.203
Serum_1	134	7.519	6.885	3.5666	6.910 to 8.129
Serum_2	134	7.968	7.505	3.599	7.353 to 8.583

Saliva_1: Cortisol levels measured in saliva samples during the first visit. Saliva_1: Cortisol levels measured in saliva samples during the first visit. Serum_1: Cortisol levels measured in serum samples during the first visit. Serum_2: Cortisol levels measured in serum samples during the second visit. SD: Standard deviation. 95% CI: 95% confidence interval for the mean cortisol concentration.

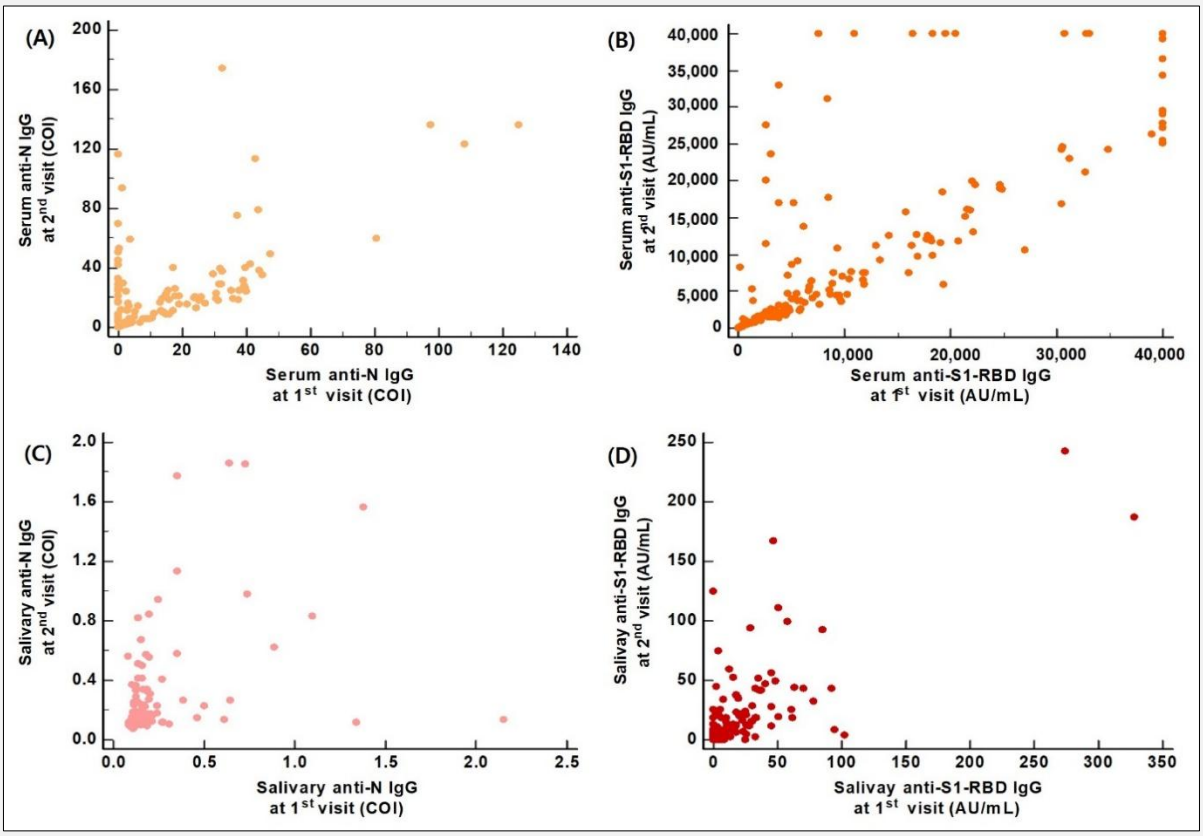

Figure 1. Correlation of SARS-CoV-2 antibody levels between two visits.

A: Serum anti-N Ab (n = 166) (r = 0.6796, p < 0.001), B: serum anti-S1-RBD Ab (n = 166) (r = 0.803, p < 0.001), C: salivary anti-N Ab (n = 166) (r = 0.415, p < 0.001), and D: salivary anti-S1-RBD Ab (n = 166) (r = 0.742, p < 0.001).

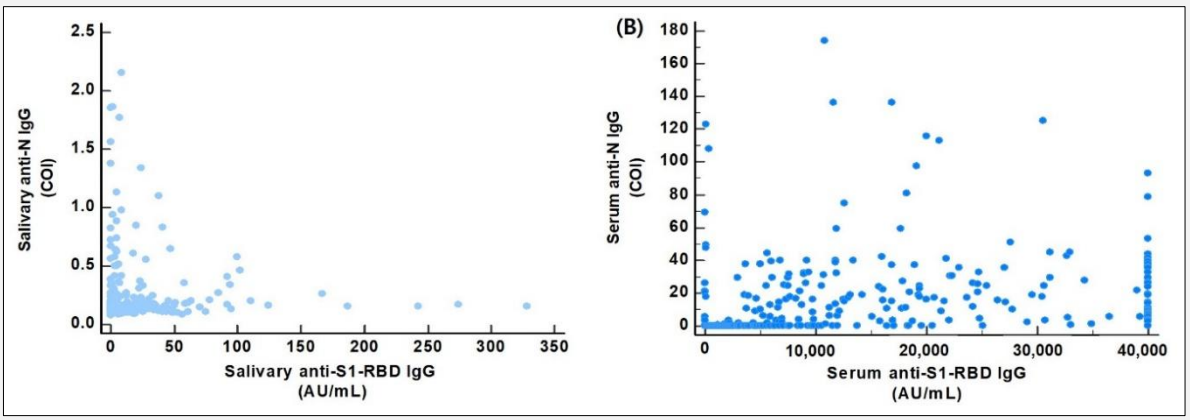

Figure 2. Correlation of SARS-CoV-2 antibody levels between anti-S1-RBD IgG and anti-N IgG titers collected at the same time.

A: Salivary samples (n = 332) (r = -0.017, p = 0.761), B: serum samples (n = 332) (r = 0.298, p < 0.001).

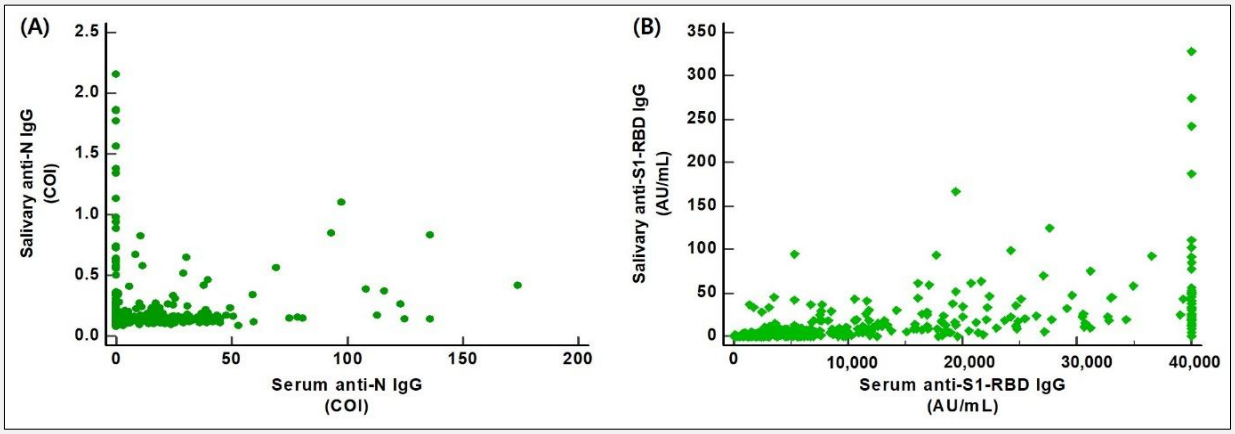

Figure 3. Correlation of SARS-CoV-2 antibody levels between saliva and serum collected at the same time.

A: Anti-N IgG (n = 332) (r = 0.0495, p = 0.368), B: anti-S1-RBD IgG (n = 332) (r = 0.543, p < 0.001).

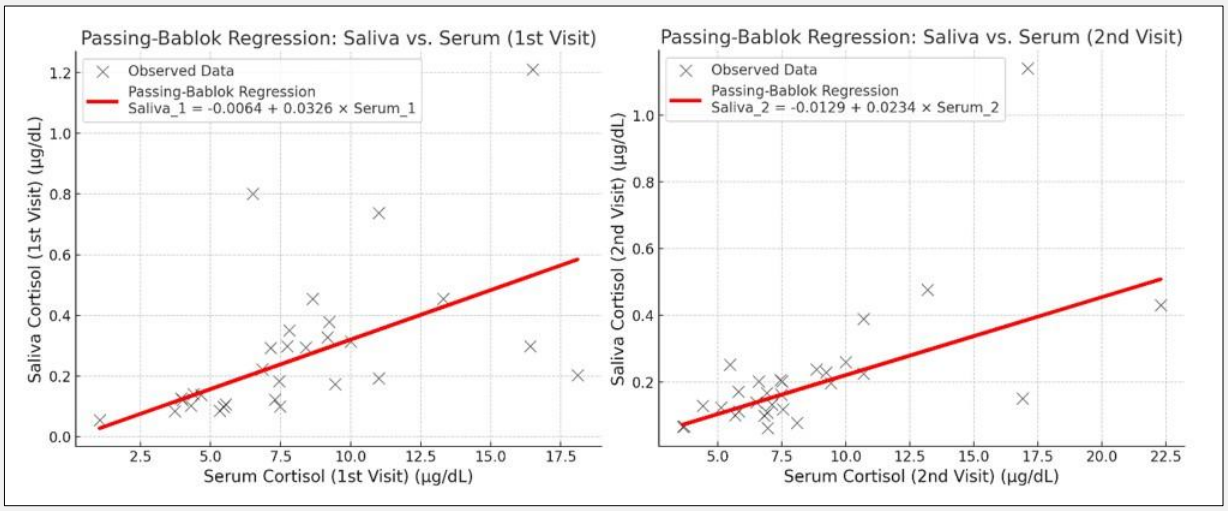

Figure 4. Regression analysis of serum and saliva cortisol levels (1st visit, 2nd visit).

tial inconsistencies in the agreement between the two measurement methods.

For the second visit, the mean relative difference was 97.59% (Figure 5), with saliva cortisol 97.59% lower than serum cortisol on average. Despite the slight increase in agreement compared to the first visit, the limits of agreement remained wide, indicating persistent variation between saliva and serum cortisol levels. These results suggest that while there is a significant correlation between serum and saliva cortisol, saliva cortisol measurements consistently underestimate serum cortisol levels. The systematic bias and variability in the Bland-Altman analysis suggest that direct interchangeability between the two measures is limited.

However, given the relatively stable ratio observed between serum and saliva cortisol, correction models

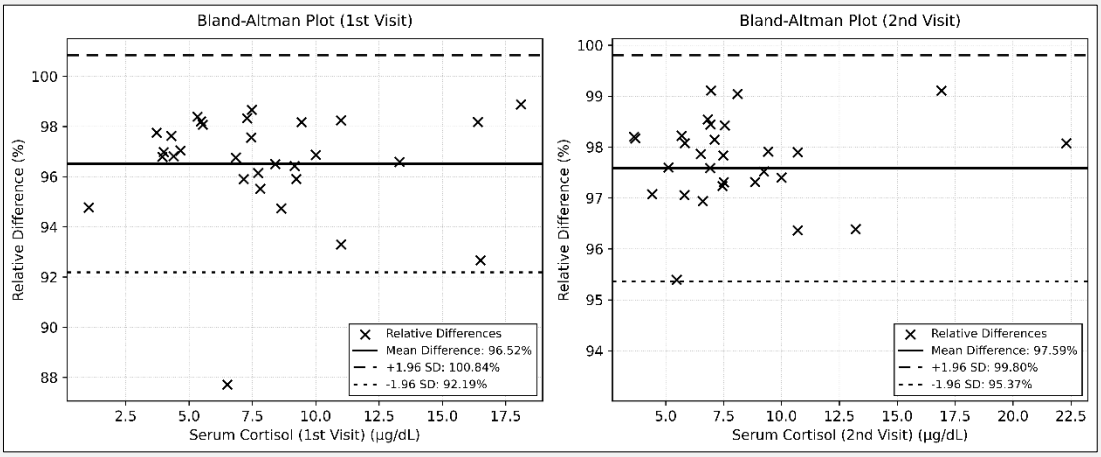

Figure 5. Bland-Altman plot of relative differences in cortisol levels (1st visit, 2nd visit).

could be developed to improve the predictive accuracy of saliva cortisol in estimating serum cortisol levels.

DISCUSSION

Given the ease of collection compared to swabs or blood, saliva emerges as an ideal specimen for widespread diagnostic use [3,4]. This study represents the first assessment of SARS-CoV-2 antibodies using saliva samples in Korea and employed automated assay by ECLIA and CLIA methods, which are commonly used in clinical laboratories.

Seropositivity for anti-S1-RBD Ab and anti-N Ab in serum was 97.6% - 98.2% and 47.5% - 58.4% during the first and second visits, respectively. A study involving a cohort of healthy individuals in Korea conducted during a similar period yielded a comparable rate of antibody positivity [16]. In another study that had started three months prior to ours, anti-S1-RBD Ab was detected in 100% of the sera of vaccinated participants [17], consistent with our study, where 99.4% of vaccinated participants exhibited serum anti-S1-RBD Ab. Notably, serologic anti-N antibody positivity was 79.3% and 3.3% in participants of the SARS-CoV-2-infected and non-infected group, respectively [17]. In our study, among the vaccinated participants, individuals with a history of SARS-CoV-2 infection exhibited higher levels of anti-N Ab, anti-S1-RBD Ab in serum, and salivary anti-S1-RBD Ab than those without a history of infection.

Prior investigations have established a gradual decline in antibody titers post-vaccination, with an additional COVID-19 infection leading to elevated antibody titers [13,16]. This observation may differ from previous findings owing to the absence of information for identified additional infections between collection intervals. Regarding saliva samples, our findings revealed poor qualitative agreement over time, in contrast to the perfect agreement observed for serum samples. Furthermore, variable correlation coefficients were observed between the quantitative anti-S1-RBD Ab and anti-N Ab at the initial and subsequent visits. Although the concentration of serum anti-N Ab was higher during the second visit, no significant variation was observed in the quantitative outcomes of the remaining antibodies.

Assays targeting antibodies against the S and N proteins, which are the most immunogenic proteins of SARS-CoV-2, are employed in the serological measurement of SARS-CoV-2 antibodies. The envelope of SARS-CoV features a spike (S) protein, which exhibits potential neutralizing effects in laboratory settings. The presence of anti-S antibodies may serve as a reliable marker for assessing the efficacy of immune responses, including vaccine efficiency [17-19]. Conversely, nucleocapsid (N) proteins form complexes with genomic RNA, contributing to the interaction with viral membrane proteins during viral particle formation. Anti-N antibodies exhibit more sway as a biomarker for spontaneous infections. The features of anti-S and anti-N antibodies vary based on the timing of SARS-CoV-2 infection, vaccination status, and symptom severity [17-19]. Therefore, in this study, the qualitative agreement between anti-S1-RBD Ab and anti-N Ab in serum and saliva samples was poor. Additionally, weak to no correlations were observed between the levels of anti-S1-RBD Ab and anti-N Ab in the serum and saliva samples.

Previous studies have demonstrated a strong association between the presence of antibodies against SARS-CoV-2 in both blood and saliva samples [3,5,20]. Other in-

vestigations have reported mean coefficients for Ab, averaging at 0.55 (95% CI, 0.38 - 9.73), indicating a moderate correlation ranging between weak and strong [21-27]. In our study, the agreement for anti-S1-RBD Ab between saliva and serum samples was characterized as moderate, whereas it was deemed poor for anti-N Ab. Notably, a moderately positive correlation was observed between the levels of anti-S1-RBD Ab in serum and saliva, whereas no correlation was observed between anti-N Ab levels in serum and saliva. The presence of SARS-CoV-2 antibodies in the mucosa post-natural infection could be associated with tissue-resident memory B cells, local antibody production by plasma cells derived from terminally differentiated plasma cells or short-lived plasmablasts, or antibody transudation from the blood to mucosal surfaces. Of particular significance is the notion that the neutralization of viral infection at the mucosal surface following intramuscular vaccination is predominantly attributed to the transudation of antibodies from plasma [28]. In a previous study, the correlation between plasma and saliva levels of SARS-CoV-2 anti-spike antibodies was statistically significant in vaccinated, uninfected individuals [28]. The robust correlation observed between the concentrations of SARS-CoV-2 anti-spike antibodies in the saliva and blood samples of unvaccinated, infected individuals suggests that these antibodies were produced locally rather than through transudation from the blood. A previous study revealed that the levels of anti-nucleocapsid antibodies in the saliva of unvaccinated, infected individuals did not correlate significantly with those in the plasma [28]. The observed absence of a correlation could be attributed to the progressive decrease in anti-nucleocapsid antibody levels. These discrepancies in correlation outcomes between serum and saliva samples may stem from diverse factors, including variations among reagents, sample collection methods, duration since vaccination, and duration since infection [10]. Notably, results differed significantly between studies owing to the variation in sample collection techniques. Several studies [10,29] employed dedicated containers, such as Salivette, whereas several other researchers used swab samples [12-14]. Therefore, the establishment of an appropriate standard method for collection is necessary to identify salivary antibodies.

In the context of antibody testing using saliva, it is deemed necessary to establish a distinct cutoff value compared to serum testing. An inherent limitation of using saliva as a biofluid lies in its exceedingly low antibody concentration [5], necessitating optimization of detection sensitivity. To determine the presence of anti-S1-RBD Ab in saliva samples, a cutoff setting distinct from the serum cutoff is necessary. In our study, particularly in instances where serum anti-S1-RBD Ab was potent, antibodies were identified in certain saliva samples. However, the concentration observed in these instances proved inadequate for a positive interpretation. And for most studies that used saliva samples, IgA was also concurrently evaluated simultaneously; however, this study was not included in the absence of the automated analyzer reagents, indicating a notable limitation. Although we did not test IgA titers, a study on the three immunoglobulin titers reported similar patterns [30] when comparing serum IgG, IgA, and saliva IgG titers with neutralization tests in various vaccination groups. Additionally, in cases where the antibody titer was over 40,000, the sample could not be diluted and was treated as 40,000, which may have affected the correlation analysis.

In conclusion, this study underscores the inability to substitute saliva samples with serum when measuring SARS-CoV-2 virus antibodies, even with the use of ECLIA and CLIA, two precise test reagents commonly employed in clinical laboratories. Comparison with highly sera-matched saliva cortisol demonstrates that it is essential to establish a cutoff value different from that used for serum analysis for SARS-CoV-2 antibodies. This is also crucial when introducing a noninvasive diagnostic method using saliva in a pandemic situation that requires convenient diagnostic tests for infectious diseases.

Declaration of Interest:

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

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