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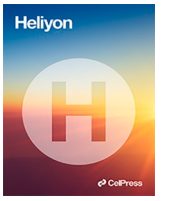

Research article

The impact of vaccine type and booster dose on the magnitude and breadth of SARS-CoV-2-specific systemic and mucosal antibodies among COVID-19 vaccine recipients

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ABSTRACT

The COVID-19 pandemic has had a major impact on global health and economy, which was significantly mitigated by the availability of COVID-19 vaccines. The levels of systemic and mucosal antibodies against SARS-CoV-2 correlated with protection. However, there is limited data on how vaccine type and booster doses affect mucosal antibody response, and how the breadth of mucosal and systemic antibodies compares. In this cross-sectional study, we compared the magnitude and breadth of mucosal and systemic antibodies in 108 individuals who received either the BNT162b2 (Pfizer) or CoronaVac (SinoVac) vaccine. We found that BNT162b2 (vs CoronaVac) or booster doses (vs two doses) were significantly associated with higher serum IgG levels, but were not significantly associated with salivary IgA levels, regardless of prior infection status. Among non-infected individuals, serum IgG, serum IgA and salivary IgG levels were significantly higher against the ancestral strain than the Omicron BA.2 sublineage, but salivary IgA levels did not differ between the strains. Salivary IgA had the weakest correlation with serum IgG ($r = 0.34$) compared with salivary IgG ($r = 0.63$) and serum IgA ($r = 0.60$). Our findings suggest that intramuscular COVID-19 vaccines elicit a distinct mucosal IgA response that differs from the systemic IgG response. As mucosal IgA independently correlates with protection, vaccine trials should include mucosal IgA as an outcome measure.

1. Introduction

SARS-CoV-2 has emerged in 2019 and caused the COVID-19 pandemic. The World Health Organization estimated that COVID-19

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was associated with 14.83 million excess deaths globally from January 2020 to December 2021 [1]. COVID-19 is also associated with a higher risk of 30-day mortality and hospital readmission when compared with influenza [2]. SARS-CoV-2 has evolved into different variants. The Omicron variant, which escape immunity induced by prior infection or vaccination, emerged in late 2021 and led to major outbreaks [3].

Humoral immunity plays an important role in the protection against SARS-CoV-2 infection. Higher levels of systemic (serum or plasma) neutralizing antibodies or SARS-CoV-2-spike-protein-specific (S-specific) IgG correlate with a better vaccine effectiveness against symptomatic infection and a lower risk of reinfection [4–6]. Omicron variant, which emerged in late 2021 and escaped neutralization by systemic antibodies elicited from prior infection or vaccination [7–10], was associated with poorer vaccine effectiveness [11]. Hence, systemic IgG or neutralizing antibodies have been considered to be a major correlate of protection, and have been used as the outcome measure in COVID-19 vaccine trials [12].

SARS-CoV-2 primarily infects and replicates in the respiratory tract, making mucosal immunity a crucial first line of defence against the virus. Secretory immunoglobulin A (IgA), derived from subepithelial plasma cells, is the major player of the respiratory tract immunity. During an acute SARS-CoV-2 infection or after COVID-19 vaccine, the level of S-specific IgA increases rapidly in the saliva [13,14]. S-specific dimeric IgA, the form present on mucosal surface, exhibits a stronger neutralizing activity than plasma monomeric IgA [15]. Several studies demonstrated that higher levels of salivary or nasal mucosal IgA correlate with lower nasopharyngeal viral load or protection from vaccine breakthrough infection [14,16–18], and the protection is independent from the level of systemic antibodies.

While numerous studies have reported that the levels of systemic neutralizing antibodies and S-specific IgG can be affected by vaccine type and booster doses [4,6,7], only a few have examined the levels of S-specific mucosal IgA after intramuscular COVID-19 vaccines [19–21]. The primary aim of the current study was to compare the levels of mucosal and systemic antibodies induced by the mRNA vaccine BNT162b2 and the inactivated whole virion vaccine CoronaVac, and to determine the impact of booster doses. We observed no significant difference in the mucosal IgA response between CoronaVac and BNT162b2 in stark contrast to the differences observed in mucosal IgG, serum IgA, and serum IgG responses. We also found that mucosal IgA has a broader binding capacity than other serum or salivary antibodies.

2. Materials and methods

2.1. Study design and study participants

This was a cross-sectional study that followed the STrengthening the Reporting of OBservational studies in Epidemiology (STROBE) guidelines. We recruited adult patients who attended the medical out-patient clinic of Queen Mary Hospital in Hong Kong between 20 June and September 8, 2022, after the Omicron BA.2 wave. Patients were eligible for inclusion if they were aged 18 years or above, and received at least 2 doses of BNT162b2 or CoronaVac. Exclusion criteria included the receipt of both BNT162b2 and CoronaVac, refusal to provide written informed consent, mental incapacity to provide written informed consent, or unable to provide sufficient volume of

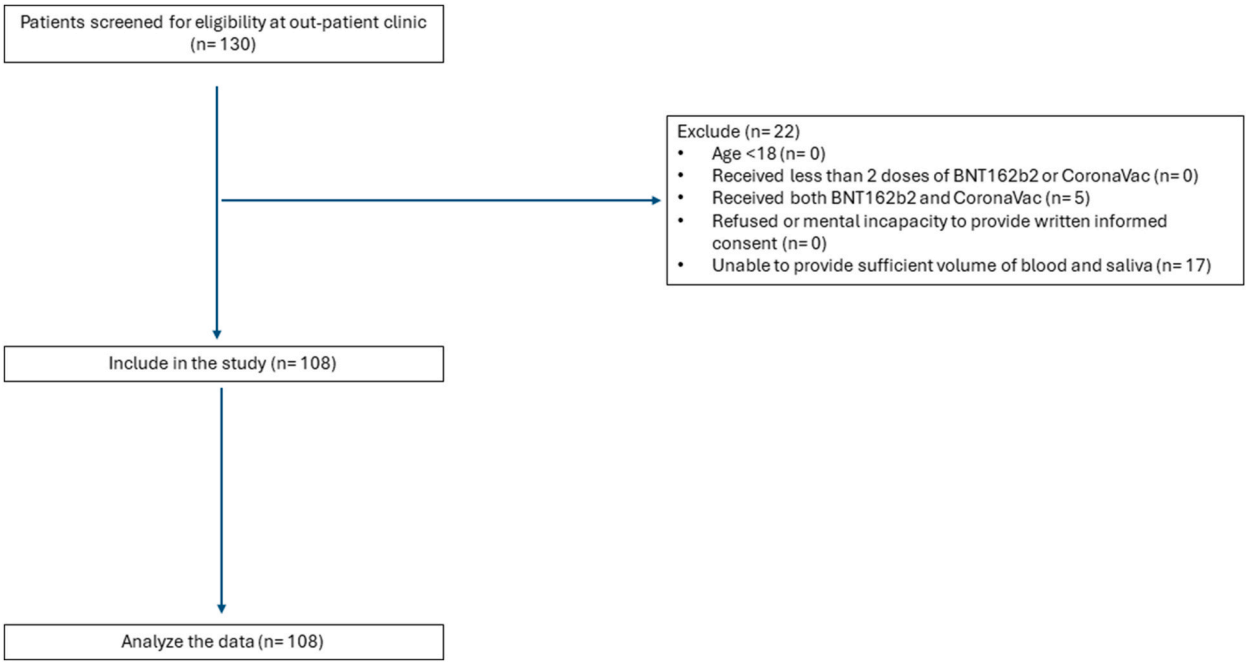

Fig. 1. STROBE flow diagram of the study.

saliva or blood (Fig. 1.).

On the day of recruitment, we collected blood and saliva specimens for testing of IgA and IgG against the SARS-CoV-2 spike protein receptor binding domain (RBD). We obtained data on vaccination history and COVID-19 infection history. Study participants were classified as having prior infection with SARS-CoV-2 if they had known positive RT-PCR or rapid antigen test results. For patients who have not received inactivated whole virion vaccine, they were also considered to have prior infection if their anti-nucleoprotein IgG (anti-N IgG) was positive.

2.2. Collection and processing of saliva specimens

Saliva specimens were collected using Salivette® (Sarstedt) according to the manufacturer's instructions. Briefly, patients were asked to place the swab in the mouth and chew for approximately 2 min to stimulate salivation. The saliva specimens were stored at −20 °C until testing. On the day of antibody testing, saliva specimens were treated with 1 % Triton X-100 at room temperature for 1 h.

2.3. Enzyme immunoassays for the detection of salivary anti-RBD IgA and IgG

Enzyme immunoassay (EIA) for the detection of saliva immunoglobulin was performed as described previously with modifications [13,22].

First, we prepared the saliva pre-absorption plate and the RBD EIA plate. For the saliva pre-absorption plates, 96-well Nunc Maxisorp™ plates (Thermo Fisher Scientific; Cat#4424–04) were coated with 200 ng/well of streptavidin (Thermo Fisher Scientific; Cat#434302) in 50 μl 0.05 M carbonate-bicarbonate buffer at 4 °C for 16 h and then blocked with blocking agent at 37 °C for 2 h.

For the RBD EIA plates, the 96-well Nunc Maxisorp™ plates (Thermo Fisher Scientific; Cat#4424–04) were coated with 100 ng/well of streptavidin as above. Then, 100 ng biotinylated recombinant SARS-CoV-2 RBD of ancestral strain (ACROBiosystems; Cat#SPD-C82E9-200 μg) or Omicron BA.2 strain (ACROBiosystems; Cat#SPD-C82Eq-200 μg) in 50 μl 1 × PBS were added. For control wells of the RBD EIA plate, PBS was added instead of biotinylated recombinant SARS-CoV-2 RBD. The RBD EIA plate was incubated at room temperature for 1 h with shaking followed by washing.

60 μl of saliva specimens were diluted to 1:2, 1:4 and 1:8, and were added to the saliva pre-absorption plate for 30 min at 37 °C for the removal of anti-streptavidin antibodies. Then, 50 μl of saliva specimens were transferred to the RBD EIA plates and incubated for 60 min at room temperature with shaking. Each plate included a positive control consisting of pooled saliva specimens from six individuals who have received BTN162b2 vaccine or were infected with SARS-CoV-2, a negative control consisting of PBS instead of saliva, and another negative control consisting of a saliva specimen tested negative for SARS-CoV-2 IgA and IgG. Antibody binding in the RBD EIA plate was performed for 1 h with shaking at room temperature. After washing, 50 μl/well horseradish peroxidase conjugated goat anti-human IgG (1:8000) and IgA (1:1000) (Thermo Fisher Scientific; Cat#A18811 and Cat#A18787) were added and incubated at room temperature for 30 min with shaking. 100 μl 3,3′,5,5′-tetramethylbenzidine single solution (Thermo Fisher Scientific; Cat#002023) was added to each well for signal development at dark for 10 min which was then stopped with 100 μl 0.3 M sulfuric acid. The optical density (OD) was read at 450 and 620 nm. For saliva specimens in which the OD value for 1:2 was >3.5, we repeated the experiments with dilutions at 1:8, 1:16 and 1:32.

The OD values of each saliva were plotted, and the area under curve (AUC) were calculated from these binding curves. To adjust for the inter-plate differences, the integrated score was calculated by dividing the AUC of each of the saliva by the AUC of the pooled saliva or serum specimen control [13]. Furthermore, we normalized the integrated score by the total IgA content in the saliva as described previously to account for saliva volume and physiological differences in IgA levels [22].

2.4. Enzyme immunoassay for the detection of serum anti-RBD IgA and IgG

The EIA for serum IgA and IgG against SARS-CoV-2 RBD was performed as for the EIA of saliva specimens, except for that the serum specimens were diluted at 1:400, 1:800 and 1:1600 for IgG, and 1:200, 1:400 and 1:800 for IgA.

2.5. Enzyme immunoassay for the detection of total IgA concentration in saliva specimens

EIA for the detection of total salivary total IgA was performed as described previously with modifications [22]. 96-well Costar microplate (Corning®; Cat#3690) was coated with 62.5 ng of goat anti-human IgA (Bethyl, Cat#A80-102A) in PBS at 4 °C for 16 h and then blocked with blocking agent at 37 °C for 2 h. Saliva specimens at a dilution of 1:1250, 1:2500 and 1:5000 were added to the wells. For control wells, PBS was added instead of saliva specimens. The plates were incubated at room temperature for 1 h with shaking followed by washing. After washing, 25 μl/well horseradish peroxidase-conjugated goat anti-human IgA (1:10000) (Bethyl, Cat# A80-102P) were added and incubated at room temperature for 30 min with shaking. 50 μl 3,3′,5,5′-tetramethylbenzidine single solution (Thermo Fisher Scientific; Cat#002023) was added to each well for signal development at dark for 10 min which was then stopped with 50 μl 0.3 M sulfuric acid. The OD was read at 450 and 620 nm.

2.6. Serum anti-N IgG assay

The level of serum IgG against SARS-CoV-2 nucleoprotein (anti-N IgG) was determined using SARS-CoV-2 IgG assay on the Alinity i system (Abbott Diagnostics, Ireland).

2.7. Statistical analysis

Statistical analysis was performed using SPSS version 26.0.0 or GraphPad Prism 9.5.1. Mann Whitney *U* test was used for assessing the difference in the antibody levels between individuals with different vaccine types (BNT162b2 vs CoronaVac) or different vaccine doses (2 vs 3 doses). Wilcoxon matched-pairs signed rank test was used in the comparison of antibody level against ancestral strain and Omicron BA.2 strain. Spearman rho was used to determine the correlation between the different salivary and serum antibodies. A P value of <0.05 was considered as statistically significant.

3. Results

3.1. Patients

We recruited a total of 108 study participants from Hong Kong Special Administrative Region between 20 June and September 8, 2022 (Table 1). The median age was 50 years old (interquartile range, 41–57), and 81.5 % (88/108) were female. 62 % (76/108) received 2 doses of COVID-19 vaccine and 38 % (41/108) received 3 doses. 71.3 % (77/108) received BNT162b2 while 28.7 % (31/108) received CoronaVac. 48.1 % (52/108) had prior infection, either confirmed by RT-PCR or rapid antigen test, or by the presence of anti-N IgG among patients who received BNT162b2.

The BNT162b2 cohort was significantly younger than the CoronaVac cohort (median age: 49 vs 54 years; P = 0.015), but there was no statistically significant difference in sex or frequency of underlying disease (Table 1).

The median time intervals between the last dose of vaccine and specimen collection were 337 days (interquartile range [IQR], 323–344 days) and 130 days (IQR, 104–145 days) for individuals with 2 doses and those with 3 doses, respectively. Among infected patients, the median time interval between the data of RT-PCR or RAT test positive date and specimen collection date was 123 days (IQR, 105–136 days). Detailed breakdown of the time intervals are shown in Table 2.

3.2. Comparison of salivary and serum IgA and IgG titers between BNT162b2 and CoronaVac recipients

First, we compared the binding antibody titers against the SARS-CoV-2 spike protein RBD between patients with different vaccine types (Fig. 2). In order to minimize the confounding effect of the number of vaccine doses and prior infection, we specifically compared patients with the same number of vaccine doses and their prior infection status. For non-infected individuals, there were no significant differences in the levels of salivary IgA and salivary IgG between BNT162b2 and CoronaVac, but the levels of serum IgA (2 doses: P = 0.0054; 3 doses: P = 0.0062) and serum IgG (2 doses: P = 0.0108; 3 doses: P = 0.0205) were significantly higher for BNT162b2 group than those of CoronaVac group. For individuals with prior infection, there were no significant differences in the levels of salivary IgA and serum IgA between BNT162b2 and CoronaVac, but the levels of salivary IgG (2 doses: P = 0.0454; 3 doses: P = 0.0151) and serum IgG (2 doses: P = 0.0126; 3 doses: P = 0.0151) were significantly higher for BNT162b2 than those of CoronaVac.

Next, we compared the antibody titers between patients with 2 doses or those with 3 doses (Fig. 2). For non-infected individuals, there were no significant differences in the salivary IgA, salivary IgG and serum IgA between 2 doses and 3 doses. However, individuals with 3 doses had significantly higher levels of serum IgG than those with 2 doses (BNT162b2: P = 0.0098; CoronaVac: P = 0.0042). For individuals with prior infection, the levels of serum IgG were significantly higher for individuals with 3 doses than those with 2 doses (BNT162b2: P = 0.0014; CoronaVac: P = 0.0357), but the level of salivary IgG was only significantly higher for 3 doses than 2 doses only for BNT162b2 recipients (P = 0.0266).

Table 1
Baseline characteristics of patients.

	CoronaVac (n = 31)	BNT162b2 (n = 77)	P value
Demographics			
Median age in years (interquartile range)	54 (45–60)	49 (36–56)	0.015
Female	27 (87.1)	61 (79.2)	0.421
Chronic comorbidities			
Hypertension	7 (23.3)	10 (13.0)	0.240
Cardiovascular disease	3 (9.7)	2 (2.6)	0.141
Lung disease	1 (3.2)	2 (2.6)	1.000
Liver disease	5 (16.1)	20 (26.0)	0.323
Kidney disease	0 (0)	0 (0)	N/A
Diabetes mellitus	0 (0)	3 (3.9)	0.556
Malignancy	4 (12.9)	3 (3.9)	0.103
Thyroid disorders	2 (6.5)	0 (0)	0.080
Autoimmune disease	0 (0)	2 (2.6)	1.000
Prior COVID infection	8 (25.8)[a]	44 (57.1)[a]	0.005
Number of vaccine doses			
2	18 (58.1)	49 (63.6)	
3	13 (41.9)	28 (36.4)	

[a] Out of the 52 patients who were considered to have prior infection, the date of infection was known for 33 patients, and they were infected between February and July 2022.

Table 2
Interval between vaccine doses.

Vaccine regimen	Median number of days between the last dose of vaccine and specimen collection (IQR unless specified otherwise)	Median number of days between infection and specimen collection[a] (IQR unless specified otherwise)
All		
2-dose-BNT162b2 (n = 49)	341 (330–345)	N/A
2-dose-CoronaVac (n = 18)	328 (313–337)	N/A
3-dose-BNT162b2 (n = 28)	132 (109–146)	N/A
3-dose-CoronaVac (n = 13)	126 (86–146)	N/A
Non-infected		
2-dose-BNT162b2 (n = 23)	343 (335–351)	N/A
2-dose-CoronaVac (n = 15)	329 (313–337)	N/A
3-dose-BNT162b2 (n = 10)	139 (126–162)	N/A
3-dose-CoronaVac (n = 8)	128 (66–149)	N/A
Previously-infected		
2-dose-BNT162b2 (n = 26)	339 (328–344)	130 (114–138)
2-dose-CoronaVac (n = 3)	321 (250–336)	150 (99–162)
3-dose-BNT162b2 (n = 18)	121 (106–139)	116 (104–133)
3-dose-CoronaVac (n = 5)	103 (86–161)	40 (20–122)

[a] Only for individuals with known date of infection.

3.3. Difference in the breadth of IgA and IgG in saliva and serum specimens

To assess the breadth of the antibodies, we compared the antibody levels against the ancestral strain and the Omicron BA.2 strain (Fig. 3). For non-infected individuals, there were no significant differences in the levels of salivary IgA between BA.2 and ancestral strain. However, for individuals with prior infection, the level of salivary IgA was significantly higher for ancestral than that of Omicron BA.2 ($P = 0.0110$). The levels of salivary IgG, serum IgA and serum IgG were significantly lower against Omicron BA.2 than those against the ancestral strain for both infected and non-infected individuals ($P < 0.0001$ for all comparisons).

3.4. Correlation between IgA and IgG in saliva and serum specimens

Next, we determined the correlation between the levels of different antibodies (Fig. 4). The correlation between salivary IgA and serum IgG ($r = 0.34$) was poorer than the correlation between salivary IgG and serum IgG ($r = 0.63$) and between serum IgA and serum IgG ($r = 0.60$).

4. Discussion

In this study, we found that S-specific salivary IgA induced by COVID-19 vaccine with or without prior infection is distinct from serum IgG, salivary IgG, and serum IgA. Although BNT162b2 recipients displayed higher levels of S-specific serum IgG compared to CoronaVac recipients, and individuals who received booster doses exhibited higher levels of serum IgG than those with two doses, the levels of S-specific salivary IgA were not influenced by vaccine type or booster doses. Furthermore, while the levels of serum IgG against Omicron variant was significantly lower than those against the ancestral strain, there was no significant differences in the levels of salivary IgA between ancestral and Omicron strains among individuals with no prior infection.

Previous studies have shown that the levels of systemic neutralizing antibody and S-specific IgG induced after CoronaVac were considerably lower than those induced by BNT162b2 vaccine [5]. Additionally, the systemic SARS-CoV-2 specific T cell response was more robust after BNT162b2 than those for CoronaVac [23]. Consistent with the serum antibody and T cell results, the vaccine efficacy of CoronaVac vaccine against symptomatic COVID-19 was much lower than those of BNT162b2 vaccine during the phase 3 randomized controlled trials [24,25]. However, despite the much poorer systemic neutralizing antibody response against the Omicron variant after CoronaVac vaccine when compared to those with BNT162b2 vaccine [7], the vaccine effectiveness against Omicron variant infection was similar between CoronaVac and BNT162b2 vaccine recipients [23]. Our findings of the similarity in S-specific mucosal IgA levels between CoronaVac and BNT162b2 recipients, as well as the enhanced breadth of S-specific mucosal IgA, may partially account for the comparable vaccine effectiveness observed despite a substantial difference in systemic antibody response.

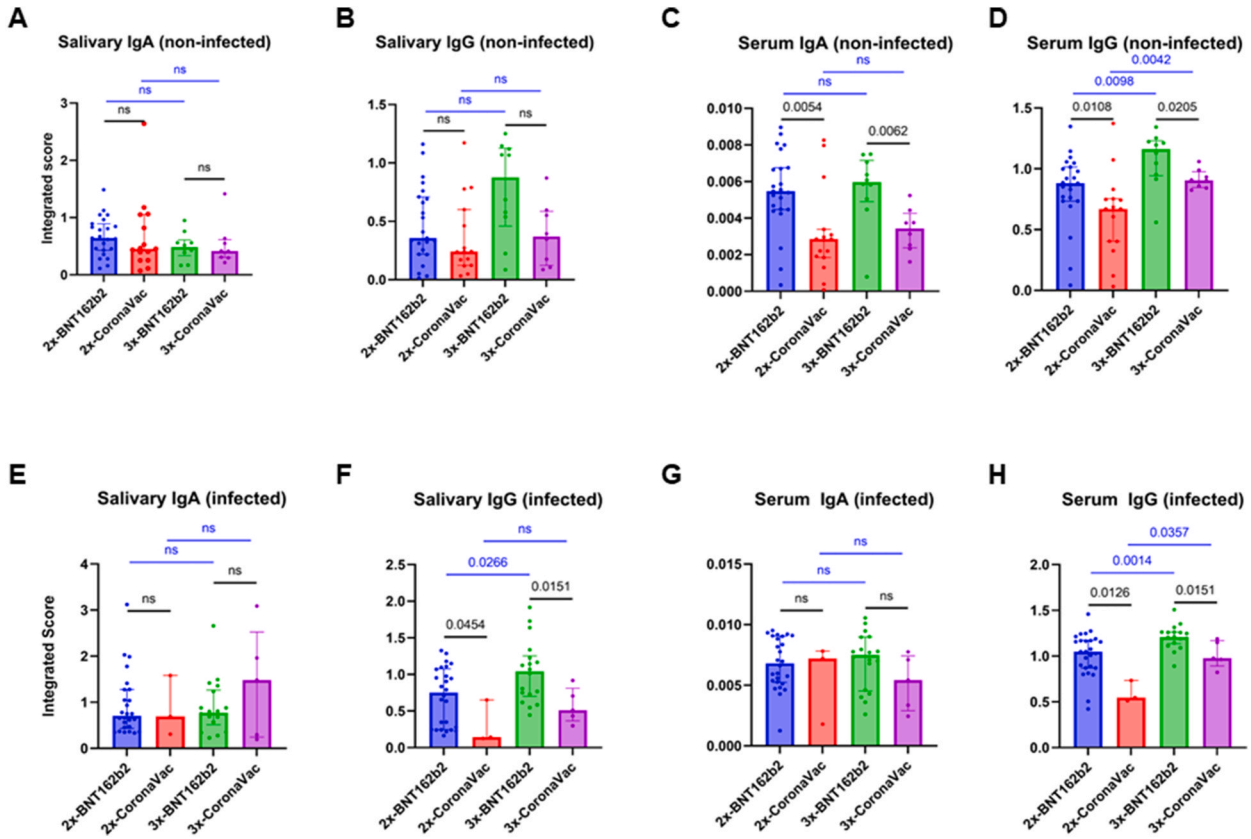

Fig. 2. Comparison of the antibody levels among individuals with different vaccine types and vaccine doses. The antibody levels against the SARS-CoV-2 ancestral strain spike protein receptor binding domain were determined using an enzyme immunoassay for non-infected individuals (A: salivary IgA; B: salivary IgG; C: serum IgA; D: serum IgG) and infected individuals (E: salivary IgA; F: salivary IgG; G: serum IgA; H: serum IgG). Mann-Whitney *U* test was performed for each comparison (BNT162b2 vs CoronaVac; 3 doses vs 2 doses). Each bar represents the median and interquartile range, and each dot represents the integrated score for each study participant. The number of patients in each group is shown in Table 2.

Our findings regarding the salivary IgA levels among CoronaVac recipients are consistent with those of Ortega et al., who also showed that salivary IgA could be detected in CoronaVac recipients without prior infection [19]. This is in contrast with the study by Chan et al., which could not detect IgA in nasal epithelial lining fluid [21]. Furthermore, while we did not find any significant difference in the salivary IgA levels between the CoronaVac and BNT162b2 recipients, Chan et al. reported significant difference between the two groups. There may be several factors contributing to the discrepancy between our findings. First, Chan et al. used nasal epithelial lining fluid, while we tested saliva specimens. Second, there was a difference in the design of the EIA. Chan et al. uses a commercially available EIA (Euroimmun). We followed the protocol of Isho et al., in which the plate was first coated with streptavidin before adding the RBD coating antigen. Anti-streptavidin antibodies were removed by first adding the saliva to streptavidin-coated plate without RBD [13]. This method augmented the titer of S-specific salivary IgA, while removing non-specific IgA antibodies.

Booster doses have been shown to improve systemic antibody response [6,26]. However, booster doses did not further increase salivary IgA antibody response in our current study. In a previous study, Sheikh-Mohamed et al. showed that the administration of the second dose of vaccine did not boost the saliva IgA level [14]. Furthermore, Ortega et al. showed that those with two doses of vaccine plus infection had similar IgA as those with 2 doses of vaccine only [19].

The Omicron variants differ in the S protein, which significantly affect the binding of systemic antibodies. Previous studies demonstrated that serum antibodies elicited by natural infection or immunization are much less active in neutralizing spike variants, especially Omicron variant, than previous strains [7,9]. Here, we showed that there was no significant difference in the levels of salivary IgA between Omicron BA.2 for both non-infected individuals. Previous studies have shown that first generation COVID-19 vaccines were also effective against Omicron variants despite severe immune escape as determined by serum neutralizing activity or monoclonal IgG. Mucosal IgA may have played a role in the protection against these variants.

We found that the differences in salivary IgG between BNT162b2 and CoronaVac recipients, and between 2 and 3 dose of vaccines, were more similar to those of serum IgG. The correlation between salivary IgG and serum IgG was also stronger than the correlation between salivary IgA and serum IgA or the correlation between salivary IgA and serum IgG. This concurs with the results from previous studies which showed poor correlation between mucosal IgA and systemic antibodies [16,27]. This is likely because salivary IgG

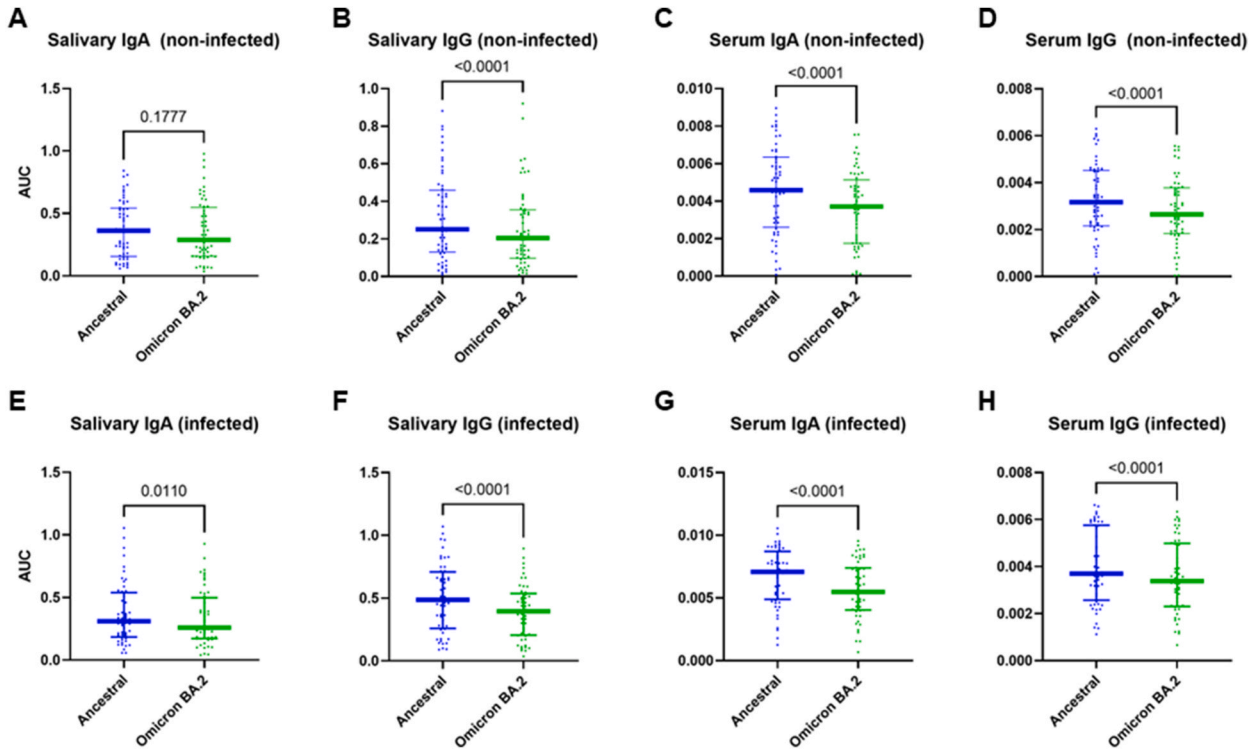

Fig. 3. Comparison of the antibody levels against the spike protein receptor binding domain of ancestral strain and Omicron BA.2 sublineage. Panels A, B, C, and D denote antibody levels of salivary IgA, salivary IgG, serum IgA, and serum IgG, respectively, in non-infected patients, while panels E, F, G, and H represent the same antibody titers in infected patients. Horizontal bar represents the median and interquartile range, and each dot represents the area under curve (AUC) for each study participant. Wilcoxon matched-pairs signed rank test was used in the comparison of antibody level against ancestral strain and Omicron BA.2 strain.

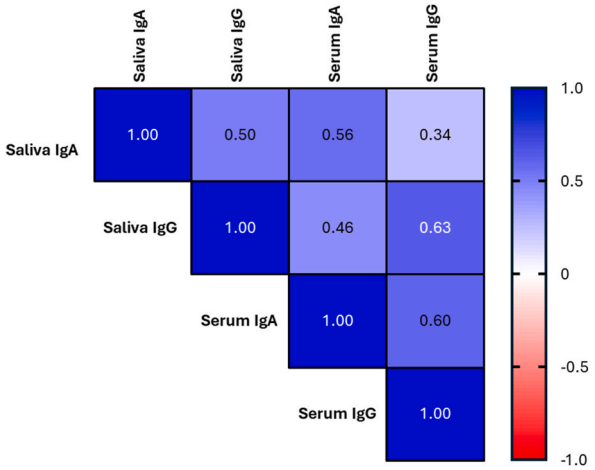

Fig. 4. Correlation between salivary IgA, salivary IgG, serum IgA and serum IgG levels.

originates from serum IgG that is transported into the mucosa.

In this study, we have taken several precautions to minimize the effect of confounding factors. First, since natural infection can induce mucosal IgA and IgG response [20,22], we separated our analysis into patients who have prior infection and those without prior infection. Second, since the IgA level can vary according to diurnal variation and other factors, we have used the total IgA level for normalization.

Mucosal vaccine has been advocated as a strategy to improve mucosal antibody response, especially for mucosal IgA [28–30]. Mice receiving mucosal vaccine have higher mucosal IgA levels than those with receiving intramuscular vaccine [31]. COVID-19 intranasal

vaccines have been approved in China, Russia, India and Iran [32]. However, in a phase 1 trial evaluating the intranasal vaccine ChAdOx1 nCoV-19, antigen-specific mucosal IgA was detected in only a minority of participants [33]. Hence, a better understanding of the mucosal humoral immunity is required for the development of more immunogenic intranasal vaccines.

There are several limitations in this study. First, some patients with prior infection may have been misclassified as non-infected because they were not tested at the time of acute infection. This limitation was addressed by using anti-N antibody to determine prior infection among patients who have received BNT162b2; however, this method is not suitable for patients who have received CoronaVac, which contains whole virions. Second, the interval between the last dose of vaccine and specimen collection was shorter for individuals who have received 3 doses than those who have received 2 doses. Given that antibody titer wanes over time [34], the difference in antibody titer between 2 and 3 doses may be related to antibody waning. Nonethelss, no difference in salivary IgA levels was observed between individuals who received 2 and 3 doses, regardless of prior infection status. Third, we did not assess the effect of the fourth dose of vaccine (second booster) or the effect of bivalent vaccine. Fourth, we did not assess the mucosal T cell immunity, which may also correlate with protection [35]. Fifth, this is a single center study in Hong Kong conducted in 2022, and the results may not be generalizable to other populations or in different time periods as prior infection may affect these results. Finally, since this is a cross sectional study, the timing of saliva collection relative to last vaccine dose varies.

In conclusion, unlike serum IgG which has been frequently used in clinical studies to assess immune response, we did not observe significant differences in the levels of mucosal IgA between CoronaVac and BNT162b2 recipients. Since mucosal IgA plays an important role in the protection against infection [17], future vaccine or infection studies should include mucosal IgA titer as one of the outcome measures. Further studies are also required to determine the relative contribution of mucosal IgA and serum IgG in the protection from infection.

Ethical statement

This study was reviewed and approved by the Institutional Review Board of the University of Hong Kong/Hospital Authority of Hong Kong West Cluster (HKU/HA HKW IRB) (Reference number: UW 13–265 [13-Sep-2022] and UW 21–214 [22-Mar-2021]). Written informed consent was obtained from all study participants.

Data availability statement

All data have been deposited in Mendeley Data at https://data.mendeley.com/preview/m43ztf9mxh.

CRediT authorship contribution statement

Hoi-Wah Tsoi: Writing – review & editing, Writing – original draft, Validation, Methodology, Formal analysis, Data curation, Conceptualization. **Miko Ka-Wai Ng:** Writing – review & editing, Validation, Methodology, Formal analysis, Data curation, Conceptualization. **Jian-Piao Cai:** Writing – review & editing, Methodology, Data curation. **Rosana Wing-Shan Poon:** Writing – review & editing, Methodology, Data curation. **Brian Pui-Chun Chan:** Writing – review & editing, Methodology, Data curation. **Kwok-Hung Chan:** Writing – review & editing, Methodology, Data curation. **Anthony Raymond Tam:** Writing – review & editing, Resources. **Wing-Ming Chu:** Writing – review & editing, Resources. **Ivan Fan-Ngai Hung:** Writing – review & editing, Validation, Resources, Formal analysis, Conceptualization. **Kelvin Kai-Wang To:** Writing – review & editing, Writing – original draft, Validation, Supervision, Software, Project administration, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

IFHN and KKWT report collaboration with Sinovac, Sinopharm and Wantai BioPharm. IFHN and KKWT are involved in the study of an intranasal DelNS1-nCoV-RBD COVID-19 vaccine. IFNH received payment from M.S.D. for speaking at the COVID-19 Regional Expert Input Forum 2021; is on the advisory board of Pfizer on COVID-19 management in 2022; was on the advisory board of Gilead on evolving treatment landscape in COVID-19 in 2021 and also on the advisory board of AstraZeneca and Fosun on the prevention of COVID-19 infection in 2022. Other authors declare no competing interests.

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