ORIGINAL ARTICLE

# Humoral response to SARS-CoV-2 vaccines among healthcare workers in a tertiary hospital in Malaysia

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## ABSTRACT

**Introduction: Healthcare workers (HCWs) were among the first to be fully vaccinated against SARS-CoV-2. However, the antibody responses to the vaccines and potential decline among Malaysian HCW are still unclear. The objective of this study is to follow-up anti-S antibody levels among HCW vaccinated with mRNA vaccine (BTN162b2) and inactivated vaccine (CoronaVac).**

**Materials and Methods: Plasma samples were collected pre-vaccination, 2 weeks and 6 months post-vaccination and tested for total immunoglobulin levels using ELISA method.**

**Results: A small percentage of HCW (2.2%, 15/677) had elevated anti-S antibody levels in their pre-vaccination plasma samples (median 20.4, IQR 5.8), indicating that they were exposed to SARS-CoV-2 infection prior to vaccination. The mRNA vaccine significantly increased anti-S levels of both previously infected and uninfected individuals to saturation levels (median 21.88, IQR.0.88) at 2 weeks post-second dose of the vaccine. At 6 months post-vaccination, the antibody levels appeared to be maintained among the recipients of the mRNA vaccine. However, at this time point, anti-S antibody levels were lower in individuals given inactivated vaccine (median 20.39, IQR 7.31, n=28), and interestingly, their antibody levels were similar to anti-S levels in pre-vaccination exposed individuals. Antibody levels were not different between the sexes.**

**Conclusion: Anti-S levels differ in individuals given the different vaccines. While further study is required to determine the threshold level for protection against SARS-CoV-2, individuals with low antibody levels may be considered for boosters.**

**KEYWORDS:**
*Anti-S COVID-19 antibody; healthcare workers; vaccination; mRNA vaccine; inactivated vaccine*

## INTRODUCTION

Healthcare workers (HCWs) were among the priority groups to be given vaccination against SARS-CoV-2. HCW are at-risk groups and prevalence of COVID-19 infection has been widely reported. A systematic review of 97 studies estimated the prevalence of SARS-CoV-2 infection was 11% (95% confidence interval (CI): 7, 15) and 7% (95% CI: 4, 11) based on molecular or serology tests, respectively.[1] Figures varied widely even within the same country in the United States, as 3.22% was reported in Seattle, Washington[2] while 57.06% in New York.[3] Vaccination among HCW in Malaysia was initiated on 24 February 2021 with the mRNA vaccine, BNT162b2 (Pfizer-BioNTech, US) followed by inactivated severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) vaccine CoronaVac® (Sinovac Biotech, China). Both are two dose vaccines, the second dose is given 2 weeks later. Clinical trials have shown an overall efficacy of 94.6% was achievable for BNT162b2 but figures varied from 50.38% to 91.25% for CoronaVac.[4]

General population studies detected significantly increased anti-spike SARS-CoV-2 antibodies, particularly after the second vaccination dose. Seroconversion was found in at least 99% of participants after the second dose vaccination with ChAdOx1 or BNT162b2.[5] The levels were even higher in individuals with previous COVID-19 infection.[5,6] Similar results were obtained with CoronaVac® with coverage of 97–99.4% response rate 28 days after the second dose vaccination of healthcare workers.[7,8] Serum antibody levels were maintained at positive levels for up to at least 6 months.[9,10] Comparative studies showed BNT162b2 induced higher levels of SARS-CoV-2 compared to ChAdOx1 levels[5,6] and CoronaVac.[10]

After an initial high, however, a decline in antibody levels was observed after receiving the second dose. Individuals provided with BNT162b2 vaccine demonstrated a substantially decreased humoral response.[11] Mean serum

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levels continued to decrease with times 10 at an estimated average of -60.05 BAU/ml with every 100 days.[6] There was no evidence that rates of antibody decline flattened over time where subjects were followed up to 119 days after second vaccination.[5]

On the one hand, the decline in IgG levels over time is expected since this occurs for all other vaccinations. However, there remain concerns about how long these antibodies remain reasonably effective[6] in addition to questions on adequate protection against mutant variants and variation in the efficacy of vaccines. Thus, the need to consider providing a booster dose is to be initiated 6 months following the completion of the second dose. Other considerations include increased adverse reactions and burnout among HCW with continued vaccination of the masses.[12,13]

The SARS-CoV-2 vaccines have been crucial in reducing the number of COVID-19 fatalities that have paralysed hospitals worldwide. Nevertheless, follow-up on seroconversion status is important as antibody levels are known to wane over time. The findings from serial measurements of antibody levels are important to policymakers, in deciding appropriate actions in terms of vaccination schedules to ensure long-term health and safety are maintained, including among HCWs.

The aim of this study was to determine anti-S antibody levels among HCWs before vaccination, 2 weeks after the first dose of vaccination and 6 months after the second dose of vaccination with the COVID-19 vaccines.

## MATERIALS AND METHODS

*Study Design*

This was a prospective longitudinal cohort study conducted between May 2020 and December 2021. The participants were followed up until after the second vaccination with the COVID-19 vaccine.

*Subjects*

Study population was HCWs. Sampling population was HCWs in a tertiary teaching hospital in Malaysia. HCWs in this study were defined as individuals who work at this hospital, comprising of formal employees as well as medical and health sciences students. The distribution of the HCWs at this hospital was as follows: 18% were physicians, 27% were nurses or nurse aids, 21% were paramedical personnel, and 34% were in the non-medical areas such as administration and logistics. Sampling frame was obtained from the hospital's human resource unit. Minimum sample size required after anticipating 15% non-response rate was 695 participants. Sampling method was simple random sampling. The sampling frame was the numbered list of names of individuals who worked at this hospital obtained from the management office. The Microsoft Excel random number generator was used to provide the random numbers to select the participants.

Participants were eligible for inclusion in the study if they were Malaysian, aged 18 years or more, worked at the study location and received two doses of COVID-19 vaccine at the study location. Participants were excluded if they were pregnant, planning to get pregnant, breast-feeding, was less than 2 weeks after surgery/vaccination/body piercing, diagnosed with acute diseases or malignancy, not vaccinated against COVID-19, or only received a single dose COVID-19 vaccination.

This study's protocol was reviewed and approved by the Ethics Committee for Research Involving Human Subjects Universiti Putra Malaysia [JKEUPM-20201-197] and the Clinical Research Unit of the hospital. The participants provided their written informed consent to participate in this study.

*Measurements*

Participants who agreed to participate in the study were invited to provide peripheral blood samples before the first COVID-19 vaccine dose, 2 weeks after the second COVID-19 vaccine dose and 6 months after the second COVID-19 vaccine dose. These pre- and post-vaccination blood samples were tested for antibodies against SARS-CoV-2 receptor-binding domain (referred to as "anti-S antibody" in the subsequent paragraphs).

The total immunoglobulin detection kit (WANTAI SARS-CoV-2 Ab ELISA, Beijing Wantai Biological Pharmacy Enterprise, China) was used. The kit contained microwell strips pre-coated with a recombinant receptor-binding domain of SARS-CoV-2 spike protein. It has a sensitivity of 94.5% (293/310) and specificity of 100% (333/333). The protocol used was according to the manual provided in the kit by the manufacturer.

The results were calculated by relating each specimen absorbance (A) value to the Cut-off value (C.O.) of the plate and reported as OD ratio, according to the manufacturer's instruction.

*Statistical Analysis*

Shapiro-Wilks test demonstrated that data were not normally distributed. Independent Kruskal–Wallis test was used to test statistically significant differences between groups while pairwise comparisons were compared between two groups.

## RESULTS

*Subjects*

Health care workers (HCW) from Hospital Pengajar, Universiti Putra Malaysia (HPUPM) were recruited for this study. A total of 666 HCWs provided pre-vaccination blood samples and almost 70% (466/666) provided a second sample 2 weeks post-vaccination. These HCW received the mRNA vaccine (BTN162b2). Majority of the sample was female. The participants' ages were similar. A smaller number (N=104) also provided a third sample 6 months after the second dose of vaccination. In addition, at 6 months post-vaccination, blood samples were also obtained from a subgroup of HCW (N=28) who completed two doses of the inactivated vaccine (CoronaVac). The summary of the results is presented in Table I.

*Anti-S antibody levels*

Among the pre-vaccinated samples, 2.2% (15/666) had positive levels of anti-S antibody, suggesting previous exposure to SARS-CoV-2 infection. In this group of

**Table I: Healthcare workers' characteristics**

| | Pre-vaccine | 2 weeks post-vaccine mRNA vaccine | 6 months post-vaccine | |
|---|---|---|---|---|
| | | | mRNA vaccine | Inactivated vaccine |
| **Total, N** | 666 | 466 | 104 | 28 |
| **Sex,** N(%) | | | | |
| Male | 183 (27.5%) | 121 (26.0%) | 25 (24.0%) | 18 (64.3%) |
| Female | 483 (72.5%) | 345 (74.0%) | 79 (76.0%) | 10 (35.7%) |
| **Age,** median (range) | | | | |
| Male | 31 (23–59) | 31 (23–59) | 30 (24–42) | |
| Female | 31 (22–59) | 31 (22–59) | 30 (24–59) | |

**Table II: Anti-S antibody levels at pre-vaccination and post-vaccination among HCW who were naturally infected and uninfected**

| | Natural infection | | Uninfected | | | |
|---|---|---|---|---|---|---|
| | Pre-vac | 2 weeks post-vac mRNA vaccine | Pre-vac | 2 weeks post-vac mRNA vaccine | 6 months post-vac mRNA vaccine | 6 months post-vac Inactivated vaccine |
| Total, N | 15 | 13 | 651 | 453 | 104 | 28 |
| M:F | 4:11 | 3:10 | 179:472 | 118:335 | 25:79 | 18:10 |
| OD ratio | | | | | | |
| Median (IQR) (range) | | | | | | |
| Total | 20.4(5.8) (1.5-21.8) | 21.9 (0.0) (21.7-21.9) | 0.0(0.00) (0.0-0.5) | 21.9(0.0) (11.9-21.9) | 21.9(0.0) (21.4-21.9) | 20.4(12.2) 1.9-21.9) |
| Male | 19.8(3.8) (12.9-21.1) | 21.9(0.1) (21.7-21.9) | 0.0(0.0) (0.0-0.1) | 21.9 (0.0) (14.5-21.9) | 21.9 (0.0) (21.4-21.9) | 20.4(12.3) (1.9-21.9) |
| Female | 20.4(7.2) (1.5-21.8) | 21.9(0.0) (21.9-21.9) | 0.0(0.0) (0.0-0.5) | 21.9 (0.0) (11.9-21.9) | 21.9 (0.0) (21.6-21.9) | 18.7(11.5) (2.7-21.9) |

Abbreviations: OD- optical density.

individuals with previous exposure to COVID-19, their median anti-S antibody level was 20.4 (IQR 5.8). At 2 weeks post-vaccination, their median anti-S antibody levels increased slightly to 21.88 (IQR 0.06). Samples of infected subjects were excluded from further analysis.

In the remaining pre-vaccination samples (n= 651), the median anti-S antibody levels were negligible (0.01, IQR 0.05), suggesting no prior exposure to COVID-19. However, at 2 weeks post-vaccination with the mRNA vaccine, the level of anti-S antibody levels in this group of samples increased to the median value of 21.9 (IQR 0.0).

A comparison of anti-S antibody levels in HCW 6 months post-vaccination showed significantly lower levels in individuals vaccinated with inactivated vaccine compared to mRNA vaccine (Figure 1). Interestingly, there was no difference in the anti-S antibody levels between individuals vaccinated with an inactivated vaccine (median 20.4, IQR 12.2) and individuals exposed to natural infection (at pre-vaccination) (median 20.4, IQR 5.8). However, a higher percentage of cases had an optical density (OD) ratio of less than 10 (n=8/28, 28.6%). Median COVID-19 antibody levels for natural infection and individuals vaccinated with an inactivated vaccine (post-6 months) were lower than mRNA-vaccinated individuals (Figure 1).

Comparison between sexes showed there was no statistical difference in the anti-S antibody levels between the sexes. These findings are summarised in Table II.

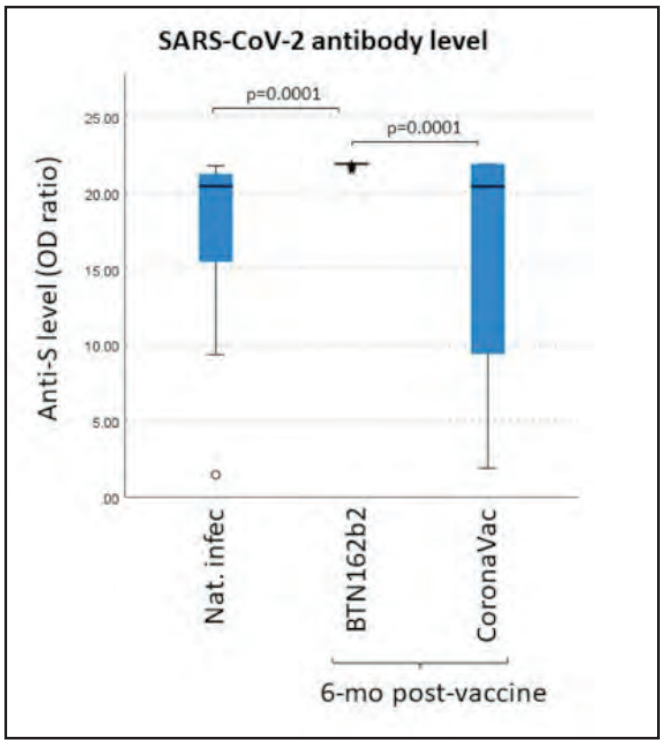


**Fig. 1:** Anti-S antibody levels in natural infection (N=15) and 6 months post-vaccination with the mRNA vaccine (N=104) and the inactivated vaccine (N=28). Pairwise comparisons followed Independent-samples Kruskal–Wallis test. $p<0.05$ considered significant. Significance values have been adjusted by the Bonferroni correction for multiple tests

## DISCUSSION

A relatively high responder rate of 96% (666/695) and 65% (453/695) than expected, was achieved for pre-vaccination and 2 weeks post-vaccination, respectively. At 6 months post-vaccination, the number of respondents had reduced to 15% (104/695). Unfortunately, higher response rate could not be obtained due to insufficient time to recruit the numbers as the period of sample collection coincided with the commencement of the booster (third) dose, which took priority. As the majority of HCW received the mRNA (BTN162b2) vaccine, only 28 respondents who received two doses of the inactivated vaccine (CoronaVac) participated in the study at the final blood sampling exercise. Nonetheless, all participants in this study showed seroconversion post-vaccination with COVID-19 vaccine.

The spike protein has been identified as the immunodominant antigen of SARS-CoV-2 virus and thus is the main candidate vaccine. Anti-viral antibodies in COVID-19 patients that inhibit and neutralise virus entry were shown to target the receptor-binding domain (RBD) of the S1 subunit.[14] The Wantai SARS-CoV-2 Ab ELISA kit is suitable to detect antibodies in response to SARS-CoV-2 vaccines. As a matter of fact, a comparison of several serological diagnostic assay kits for COVID-19, identified Wantai total immunoglobulin (Ig) kit to have best overall characteristics including to detect the presence of protective antibodies.[15] The virus neutralization test is the gold standard to demonstrate the presence of coronavirus inhibitory antibodies. A high correlation (r=0.829) with the plaque-reducing neutralizing assay (PRNT50) was achieved with the Wantai Ig kit and protective neutralising antibodies was set at cut-off point at OD ratio > 10.[15] These suggested levels of anti-RBD antibodies detected by this kit are suitable alternative markers to detect the presence of anti-SARS-CoV-2 neutralizing antibodies. A limitation of the Wantai ELISA kit is the 'semi-quantitative' format which limits the range of reading, with a maximum read at 21.9 OD ratio. This, however, was comfortably way higher than the protective antibody cut-off value.

No differences were observed in the antibody levels in sex and age. This was in contrast to waning antibody seen following peak levels during days 4 through 30 in an Israel population vaccinated with mRNA vaccine, showing substantially lower levels of antibodies among males compared to female.[11] Older age and male sex were associated with substantially lower peak antibody levels in vaccinated participants[5] as seen also for age (>35 years old) among health care workers.[16] The discrepancy in the results here may be due to the higher number of younger (71%, 322/453, <35 years old) and small number of older (1.3%, 6/453, >55 years old) participants among our 2-weeks post-vaccination HCWs.

A prevalence of 2.2% (15/666) anti-S antibody positivity of responders in the pre-vaccination state among HCW was much lower than the estimated 7% (95% CI: 4, 11) in a meta-analysis of 97 studies.[1] A Malaysian study of 400 HCW from the National Public Health Laboratory and two COVID-19 designated public hospitals in Klang Valley between April 13, 2020, and May 12, 2020, on the other hand, detected zero prevalence even though a majority claimed exposure in the past month within respective workplaces.[17] Nonetheless, these findings may be explained by the exclusion of HCW previously confirmed for COVID-19 from the study population, as the aim of that study was to identify cases that were missed. Additionally, the low prevalence was due to high adherence to PPE. As for the current study, the low prevalence of anti-S antibody positivity in the pre-vaccination state among HCW could be due to the timing of data collection: it was conducted when the hospital had yet to receive COVID-19 cases.

Being infected with COVID-19 can result in several benefits for the individual. Previous infection can provide protection against the risk of subsequent infections by 80·5–100%[18] specifically with an estimated 60.2 to 97.6% against the alpha variant, 85.7% (95% CI, 75.8 to 91.7) against the beta variant, 92.0% (95% CI, 87.9 to 94.7) against the delta variant, but only 56.0% (95% CI, 50.6 to 60.9) against the omicron variant.[19] Previous infection is also an advantage to individuals vaccinated with CoronaVac as anti-S antibody levels were maintained at significantly higher levels compared to vaccinated HCW without prior infection.[8]

The results of the current study showed that HCWs vaccinated with BTN162b2 reached substantially high levels at median OD ratio of 21.9, 2 weeks post-vaccination and maintained this median level for at least 6 months. This increase supports earlier studies that were reviewed in individuals vaccinated with various SARS-CoV-2 vaccines.[5,6,7,8,9,10,11] Nevertheless, comparative studies on different vaccines demonstrated variation in levels of antibodies achieved. mRNA-1273 generated higher peak levels than BTN162b2 vaccinated individuals.[16] Studies in UK[5] and Kuwait[6] demonstrated higher antibody levels in BNT162b2 than ChAdOX1. Similar to our results, Kwok et al.[10] showed higher levels following BTN162b2 than CoronaVac. The important question, however, is whether all the vaccines provided a sufficient level of protection against later infections by SARS-CoV-2 and its various mutants. The SARS-CoV-2 surrogate virus neutralisation test[14] has been used to predict vaccine efficacy. Interestingly, although antibodies from ChAdOx1 individuals were lower than BNT162b2, the mean percentages of neutralizing antibodies were at similar levels,[6] suggesting lower levels per se should not be of immediate concern. As discussed above, OD ratio >10 is the cut-off value to imply the presence of neutralising antibodies with the Wantai Ig ELISA kit used here. OD ratio median values of 21.9 achieved here suggested neutralising antibodies were highly present, particularly after complete vaccination with BTN162b2.

Various studies have reported on the decline in SARS-CoV-2 antibody levels following initial peak after second dose of vaccine. The mean antibody half-life was estimated as 79 days. While prior infection extended the half-life by 13 days, very small reductions in half-life were observed at older ages, in non-white ethnicity and in having a long-term health condition.[5]

In this study, no significant reduction in antibody levels was detected at 6 months compared to 2 weeks post-vaccination, particularly among BTN162b2 vaccinated individuals. As expected, 6 months post-vaccination antibody levels in CoronaVac vaccinated participants were significantly lower than BTN162b2. Anti-S antibodies were observed to have decreased significantly by day 42 post-vaccination compared with day 14 post-vaccination, which were then maintained at

least for 98 days post-vaccination.[8] Here, at least 28% (8/28) of HCW vaccinated with CoronaVac appeared to no longer have sufficient protective neutralizing antibodies (OD ratio < 10). This result was similar to Kwok et al.[10] where median antibody levels of CoronaVac but not BTN162b2 vaccinated individuals fell below the cut-off protective antibody levels. Furthermore, it was shown this fall occurred 4 months after vaccination.[10] In this study, it was also demonstrated that among pre-vaccinated HCW with previous infection, a percentage, 13.3% (2/15) did not have the protective levels of neutralizing antibodies. Therefore, previously infected individuals also require vaccination.

Interestingly, the study by Nam et al.[9] observed significantly lower levels of anti-SARS-CoV-2 antibodies in individuals (N=50) with higher weight (>55 kg), and BMI (>22), 6 months post-second vaccination, suggesting another group to be recommended for booster does.

The main limitation of this study is its small sample size due to the high attrition rate. The high attrition rate could also be due to the high workload hence preventing the HCWs to participate in blood-taking exercise. Nevertheless, the findings in this study addressed the study objectives. Another limitation was the different SARS-CoV-2 antibody kits that were used in different studies making comparison difficult. This could be resolved by using the WHO international binding antibody unit per ml[5,6] although thresholds set may still differ. The ELISA kit used here also limited the detection of the range of antibody levels and is better to be replaced with quantitative kits.

## CONCLUSION

There was a small percentage of HCW who were exposed to SARS-CoV-2 before the vaccination campaign started. The mRNA vaccination increased anti-S antibody levels to protective levels which were stably maintained at 6 months post-vaccination. Anti-S level at this time point was significantly lower among HCW vaccinated with the inactivated vaccinate, where more than a quarter did not have protective levels. This suggests a booster dose would benefit these individuals.

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