Research Article

Neutralising antibodies among doctors and nurses at six weeks and six months following two doses of ChAdOx1 nCoV-19 corona virus vaccine at two teaching hospitals

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Abstract

Introduction: The presence of neutralising antibodies (NAbs) indicates protection against SARS-CoV-2 infection. Sri Lankan healthcare workers (HCWs) were vaccinated with two doses of ChAdOx1 nCoV-19 corona virus vaccine three months apart as the primary course. This study was done to determine and compare the prevalence of NAbs against SARS- CoV-2 among doctors and nurses vaccinated with a primary course of ChAdOx1 nCoV-19 corona virus vaccine at 6 weeks and 6 months and to determine the co-morbidities associated with non-development of NAbs.

Methods: A prospective descriptive study was carried out at two teaching hospitals to determine the presence of NAbs in serum of doctors and nurses who were vaccinated with 2 doses of ChAdOx1 nCoV-19 Corona virus vaccine at 6 weeks and 6 months following the 2nd dose. A total of 205 serum samples were tested for presence of NAbs by an ELISA test (C Pass ™ SARS CoV -2 Neutralization Antibody Detection Kit).

Results: At 6 weeks, 115 of 128 (89.8%) had NAbs while 58 of 77 (75.3%) had antibodies after 6 months. In the comparison group which was tested at both time points (n=55), 41 of 55 (74.5%) demonstrated persistence of NAbs while 12 of 55 (21.8%) became seronegative by 6 months. Two HCWs (3.6%) did not have antibodies at 6 weeks or at 6 months. None of the comorbidities were significantly associated with non-seroconversion at 6 weeks or sero-reversion of neutralising antibodies at 6 months.

Conclusions: The majority of HCWs developed NAbs after 6 weeks and 21% became seronegative by 6 months.

Keywords: *Neutralising antibodies, ChAdOx1 nCoV-19 Corona virus vaccine, Healthcare workers*

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Introduction

SARS CoV-2 had become a global challenge with a 17.3% overall case fatality rate across China at the beginning of the pandemic during January 2020.[1] Globally, the pandemic had dramatic negative consequences on the economy where the world's collective gross domestic product decreased by 3.4% during 2020.[2] The estimated R_0 of this infection ranged from 1.5 to 6.68 according to data from 12 studies published in early 2020.[3] The virus transmits from human to human and infection resulted in high mortality rate in the critically ill. A meta-analysis revealed that hospital mortality was 25.9% and ICU mortality was 37.3%.[4] The array of clinical manifestations varied from asymptomatic, mild flu like illness, and acute respiratory distress syndrome.[3,4] Control measures like social distancing, hand hygiene and use of masks were put into place to curb transmission, but they did not seem to completely control the spread of the infection. Therefore, vaccination became a very important measure to control the disease.

Four types of vaccines were available for COVID-19. ChAdOx1 nCoV-19 corona virus vaccine is a non-replicating viral vector vaccine which has a recombinant replication defective chimpanzee adenovirus expressing the SARS-CoV-2 S surface glycoprotein. The S- protein, an envelope glycoprotein on the outer surface of the virus serves as a main target for development of antibodies following natural infection and vaccination.[5] Studies have shown that both helper and cytotoxic T cells recognize spike proteins and produce a T- cell dependent B cell response by producing antibodies.[6] Memory B cells resulting from an initial infection or vaccination are responsible for protection against reinfection. There have been several studies showing the importance of neutralising antibodies following vaccination and effective vaccination is therefore of utmost importance in controlling the SARS CoV-2 infection.[7,8,9]

Vaccine efficacy and immunogenicity is also an important feature which will indicate the long-term prevention of infection or reinfection. Vaccination programmes against COVID-19 were rolled out in many countries from December 2020 and in Sri Lanka from the end of January 2021. Sri Lankan healthcare workers (HCWs) were vaccinated with the ChAdOx1 nCoV-19 non-replicating viral vector vaccine. Vaccination with the first dose was started on 29th January 2021. The booster was given after 3 months. According to a study conducted among adults vaccinated with the Covishield vaccine, persistence of neutralising antibodies following 2 doses was around 4 weeks to six months.[8]

This study aimed to determine the presence of neutralising antibodies in health care workers following 2 doses of the vaccine at 6 weeks and at 6 months following the 2nd dose of ChAdOx1 nCoV-19 vaccine which would indicate a possibility of long-term prevention of reinfection in Sri Lankan HCWs. The study also looked for factors affecting the development of neutralising antibodies against the vaccine such as various comorbidities, and risk behaviours such as smoking, alcohol consumption, age and gender.

Methods

A descriptive cross-sectional study was carried out to determine the prevalence of neutralising antibodies among health care workers vaccinated against SARS- CoV-2 at 6 weeks and 6 months

following the primary course of vaccination at the Department of Microbiology, Faculty of Medicine, University of Kelaniya from February 2021 to February 2023 after obtaining the necessary ethical and institutional approvals.

A sample of doctors and nursing officers working at the Colombo North Teaching Hospital, Ragama, and Teaching Hospital, Kegalle, who had been vaccinated with 2 doses of ChAdOx1 nCoV-19 coronavirus vaccine 12 weeks apart was included in the study. Health care workers who had been confirmed with COVID-19 infection or who had a significant exposure to a confirmed case of COVID-19 and undergone 14-day quarantine, and those with an acute febrile illness or general ill-health at the time of collecting the blood samples for the study were excluded from the study.

The selected participants were informed of the study and consent was obtained. Blood was collected 6 weeks and 6 months after the 2nd dose of the vaccine.

A convenient sample of doctors and nurses from both hospitals who were working during the morning shift in the main clinical wards were recruited. A total of 84 doctors and nurses from the North Colombo Teaching Hospital and 66 doctors and nurses from the Teaching Hospital Kegalle were recruited. At 6 weeks, 128 and at 6 months, 22 new participants were included.

Among the HCWs who participated at 6 weeks (n=128), only 55 HCWs consented to give blood for testing at 6 months. This group was therefore considered as the comparison group (n=55) in whom the NAbs were tested at both time points to determine the persistence of antibodies at 6 months.

A total of 205 serum samples were collected for this study. At 6 weeks, 128 samples were tested and at 6 months 77 samples were tested which included 55 HCWs who were recruited at 6 weeks and 22 new HCWs.

Serum was separated and stored at -20 °C until the test was performed. An ELISA test (C Pass ™ SARS CoV -2 Neutralization Antibody Detection Kit) was performed on the serum samples according to the manufacturer's instructions. This test has 100% specificity and 95% sensitivity for detection of antibodies against SARS-CoV-2. As per kit protocol, ≥ 30% signal inhibition was taken as a positive result and the presence of neutralising antibodies indicates a protective level of antibodies.

Data was recorded in an electronic database and analysed using the Statistical Package for the Social Sciences (SPSS) version 20. Measures of central tendency were calculated for quantitative variables, and frequencies and percentages were calculated for categorical variables. To compare the results in different age groups and gender and to compare the significance of risk behaviours and comorbidities, a chi-square test was performed.

Results

At 6 weeks following the 2nd dose of vaccination, 128 HCWs and at 6 months, 77 HCWs were tested for presence of neutralising antibodies. The median age of the study population was 31-40 years. Among the HCWs who participated, 115 (76.7%) were females.

Table 1: Age and gender distribution of the participants

	Age (years)	Healthcare workers	
		No	%
Age group	21-30	25	16.6
	31-40	59	39.3
	41-50	41	27.3
	51-60	24	16
	>61	1	0.7
Gender	Male	35	23.3
	Female	115	76.7

Of the 128 HCWs, 115 (89.8%) had neutralising antibodies at 6 weeks, whereas 13 (10.2%) did not have antibodies at 6 weeks following the 2nd dose of vaccination.

This study showed a higher percentage of males (25 out of 26, 96.1%) having a neutralising antibody at 6 weeks following vaccination compared to females (89 out of 102, 87.2%). However, this difference was not statistically significant (p > 0.05).

Table 2: Neutralising antibody response at 6 weeks following vaccination according to the age of HCWs

Age group (years)	Positive antibody response (%)	Negative antibody response (%)	p value
21-30	95.4	4.6	>0.05
31-40	87.8	12.2	>0.05
41-50	82.9	17.1	>0.05
51-60	95.2	4.8	>0.05
>60	100	0	>0.05

When the antibody response at 6 weeks was compared in the different age groups, 95.4% of seroconversions were observed in the 21-30 year age group which was comparatively higher than in the other age categories (Table 2). However, this difference was not statistically significant (p>0.05). There was only one HCW in the >61-year-old age category in our study population who developed NAbs at 6 weeks following vaccination.

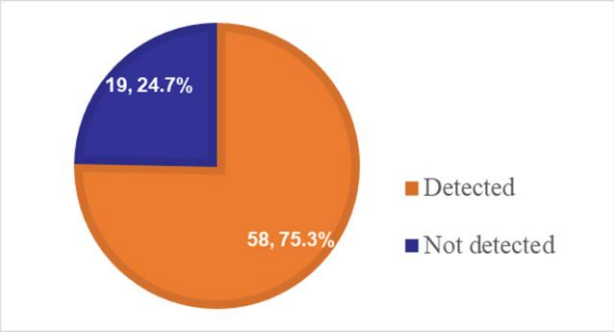

Figure 1: The prevalence of neutralising antibodies among HCWs at 6 months following vaccination (n=77)

Of the 77 HCWs tested at 6 months following 2 doses of the vaccine, 58 (75.3%) HCWs had persistent neutralising antibody responses which was not present in 24.7% (Figure 1). Similar to the response at 6 weeks, a higher percentage of males (14 out of 17, 82.3 %) had persistent neutralising antibodies compared to females (44 out of 60, 73.3 %) at 6 months following vaccination. However, this difference was not statistically significant (p >0.05).

Table 3: Neutralising antibody response at 6 months following vaccination according to the age of HCWs

Age group (years)	Positive antibody response (%)	Negative antibody response (%)	p value
21-30	87.5	12.5	>0.05
31-40	65.4	34.6	>0.05
41-50	84	16	>0.05
51-60	76.5	23.5	>0.05
>60	0	100	>0.05

Among the youngest age group (21-30 years), 87.5% had a persistent neutralising antibody response at 6 months, which is comparatively higher than the other age categories. However, the difference in the antibody response among different age groups was not statistically significant (p>0.05). The single HCW who was >61 years did not have antibodies at 6 months.

In the 53 HCWs who were seropositive at 6 weeks and retested 6 months post vaccination, 41 (74.5%) remained NAbs positive at 6 months while 12 (21.8%) became seronegative at 6 months (Figure 2).

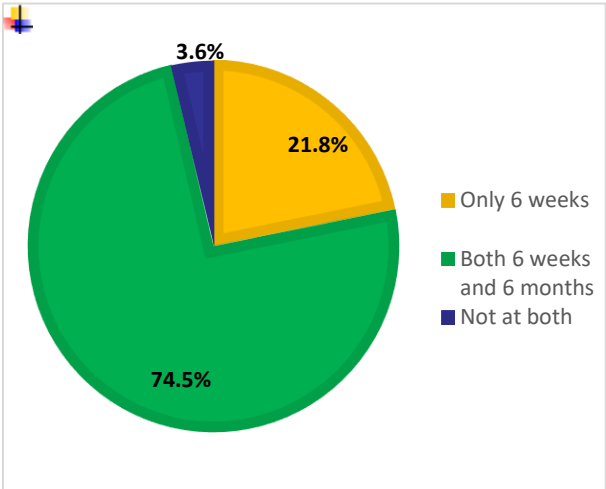

Figure 2 : Comparison of neutralising antibodies at two time points (6 weeks and 6 months) (n=55)

Among the participants who did not have NAbs either at 6 weeks or 6 months (n=30), three had diabetes, six were on treatment for various conditions during vaccination (on a course of antibiotics (n=2), on NSAIDs (n=2), on steroids (n=1), on treatment for malignancy (n=1)) and one HCW was an alcohol dependent. Among the two HCWs who did not have antibodies on either occasion, one HCW was on a course of antibiotic therapy and the other one did not have any identifiable risk factor. However, none of these factors was significantly associated with non-development or persistence of neutralising antibodies (p> 0.05).

Discussion

The current study highlights that the majority of HCWs developed NAbs by 6 weeks and 21.8% became seronegative by 6 months.

The role of the innate and adaptive immune response following vaccination and natural infection with SARS CoV-2 is well characterised in several studies.[10,11,12] The World Health Organization (WHO) and Emergency Use Listing Procedure (EUL) approved vaccines against SARS-CoV-2.[5] These vaccines have proven to produce a good neutralising antibody response and T cell response.[13,14] However, since it is known that antibody levels decrease with time, we aimed to

investigate the anti-spike neutralising antibodies at 2 time points following two doses of ChAdOx1 nCoV-19 Corona virus vaccine given at 6 weeks and 6 months.

According to a comprehensive study carried out among 139,067 individuals vaccinated with the Oxford AstraZeneca vaccine, it was noted that the antibodies against SARS-CoV-2 rose rapidly following the first vaccination, peaking 4–5 weeks after the initial dose and then gradually declined until the administration of the second dose. They found that the IgG antibody response after two doses was high in younger age groups but fell below 90% from 35 years upwards, declining to 72.7% in the oldest age group who were >75 years old . The antibodies waned after 10 weeks in the younger population, but with a quicker decline after 3-4 weeks in those aged 70+ years.[15] A study done on a Malawian population showed that the neutralising antibodies waned within 6 months post SARS-CoV-2 infection. The researchers concluded that giving a single dose of ChAdOx1 nCoV-19 Corona virus vaccine to those who had been previously infected boosted the antibody production against spike protein 2 to 3-fold.[16] Another study showed a good antibody response in HCWs following two doses of COVID vaccine with a 96.2% seroconversion rate. Ebinger *et al* showed that the neutralising antibody levels were persistent in 99.6% of HCWs up to 10 months after the initial two-dose schedule of the mRNA vaccine.[17]

Our study showed that the youngest age group had the highest seroconversion rate at both time points (at 6 weeks and 6 months) compared to the other age categories following vaccination, although this finding was not statistically significant.

Ward *et al* demonstrated that the vaccine response was better in females than males.[15] Females, younger age groups and absence of hypertension were significantly associated with higher and persistent antibody response in HCWs.[17] Females were associated with significantly higher antibody responses in another study done in HCWs vaccinated with AstraZeneca vaccine. Smoking was the only significantly associated risk factor for reduced antibody response in this study.[18]

It has been shown that some immunosuppressive conditions such as diabetes and post-kidney transplant patients had a lower antibody response following COVID-19 vaccination. The CAVEAT study demonstrated that the COVID-19 vaccines induced a weak immune response in diabetic patients with poor glycaemic control compared with non-diabetic individuals and diabetic patients with good glycaemic control.[19] Another study by Chukwu et al showed that post-kidney transplant patients had a poor response following vaccination compared to the general population.[20] However, we could not find any associated factor/risk factor significantly contributing to non-seroconversion or non-persistence of neutralising antibodies.

One limitation of our study was that we could not do a follow up antibody response at 6 months in all HCWs who were recruited at 6 weeks. This was due to the fact that the NAb ELISA kit could not be purchased due to the unavailability of the kit (as a result of the economic crisis in the country) and 35% of HCWs declined to give consent for participation. Another major limiting factor was that a pre-vaccination base-line blood sample could not be obtained in the study population to determine whether they had neutralising antibodies due to subclinical infection, as this study was designed after giving the first vaccine dose to HCWs in the 2 hospitals.

Conclusion

The majority of HCWs developed NAbs after 6 weeks and 12 HCWs (21%) showed waning of antibodies at 6 months. The youngest age group showed comparatively higher seroconversion rates and persistent antibody responses than the other age groups, although this was not statistically significant.

Declaration

Conflict of interest: The authors have no conflicts of interest to declare
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Abeykoon MM- Sample collection and processing, manuscript editing

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