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A study on seroconversion following first & second doses of ChAdOx1 nCoV-19 vaccine in central Kerala

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***Background & objectives*: Vaccination against COVID-19 induces spike protein-binding IgG antibodies, a robust correlate of protection against COVID-19. This study was undertaken to assess the humoral response after completion of both the doses of ChAdOx1 nCoV vaccine in healthcare workers (HCWs) at a tertiary care health centre in India.**

***Methods*: A cross-sectional COVID-19 vaccine-induced antibody study was conducted among HCWs. IgG antibodies against spike protein were measured at least 28 days after the first dose and the second dose of vaccination in both SARS CoV-2 naïve and recovered HCWs. Mean and median antibody titre following each dose of vaccine and its association with age, gender, co-morbidities and factors such as exercise, stress and sleep deprivation were also explored.**

***Results*: Among the 200 vaccine recipients, 91.5 per cent showed seroconversion after the first dose and 99.5 per cent after the second dose. The mean titre after the second dose was significantly higher when compared to the first dose (12.68±4.17 *vs*. 9.83±6.3, *P*=0.001). More than half (54%) had high antibody titre ≥12 S/Co (Signal/cut-off). Previous COVID-19 infection was the single most important factor influencing antibody production, where the mean titre just after a single dose [mean-17.81±5.94, median-20.5 (interquartile range [IQR]-3.7)] surpassed the titre after the second dose in SARS CoV-2 naïve individuals [mean-12.29±4.00, median-12.8 (IQR-3.7), *P*=0.001]. Furthermore, 28 per cent of vaccinees showed a reduction in titre after the second dose. The mean fall in titre was 2.25±1.40 and was more pronounced in males, the younger age group and those with previous COVID-19 infection.**

***Interpretation & conclusions*: ChAdOx1 nCov-19 vaccine after two doses elicited an excellent immune response. However, greater immunogenicity after the first dose was seen among those with previous COVID-19 infection, even surpassing the titre achieved by the second dose of vaccine in SARS CoV-2 naïve recipients. A fall in antibody titre after the second dose is a matter of concern and requires further studies.**

Key words ChAdOx1 nCoV-19 - immunogenicity - Oxford AstraZeneca vaccine - second dose - seroconversion

The COVID-19 pandemic has been a serious threat to the mankind. In this scenario, it is important to know the efficacy of the vaccines used to immunize our population. The first vaccine to be launched in India

on January 16, 2021 was ChAdOx1-nCoV vaccine . It is a modified chimpanzee adenoviral - vector vaccine, claiming efficacy of 76 per cent against symptomatic infection in recipients who took the vaccine at least 22 days apart[1]. The vaccine causes the expression of SARS-CoV-2 spike protein gene, which in turn induces host cells to produce the protein of S-antigen. This allows the body to generate an immune response and retain the information in the memory immune cells. Vaccination against COVID-19 induces spike-protein-binding IgG antibody levels and a robust correlation has been observed between antibody titre and efficacy[2]. However, it is important to note that correlation does not imply causation, and factors other than antibody responses may also play an important role in impacting efficacy.

Although some vaccines offer life-long protection, for many diseases, but immunity wanes over time[3]. This study compared the antibody titres following both the first and second doses to have a closer look at the waning effect of immunity in our setting. In the case of COVID-19, newer more transmissible variants are also a threat, compounded with the worldwide deficit in the vaccine supply chain. In this scenario, it would be worthwhile to think about a flexible immunization schedule allowing to mix and match vaccines, which could possibly trigger a more potent immune response[4] and also have an additional benefit in providing both prime and booster doses of vaccine against SARS-CoV-2 within the recommended dosage interval.

Understanding the dynamics of post-vaccine antibodies after both the first and second doses and how these differ between individuals by age, gender, co-morbidities and previous COVID-19 infection become important. To understand the immunogenicity of the ChAdOx1 nCoV-19 vaccine, a cross-sectional study was conducted on healthcare workers of a tertiary care health centre in South India to assess the seroconversion (antibody responses) after vaccination.

Material & Methods

The study was conducted at Believers Church Medical College, Thiruvalla, Kerala, India, a tertiary care centre, during February-April 2021 after obtaining clearance from the Institutional Ethics Committee (IEC 2021/04/203). Informed consent was obtained from all participants, which also included permission for blood collection at pre-determined intervals.

The study participants were 200 healthcare workers (HCWs) - doctors, nurses and supporting staff, who were enrolled for vaccination during the first three days of the first phase (January 16-February 28) of the COVID-19 vaccination drive. These HCWs were randomly selected by the district health officials through a computer-generated random sampling, from the staff list in October 2021.

Ramasamy *et al*[5] reported that seroconversion after the first dose of the ChAdOx1 nCoV-19 vaccine was 91 per cent. Hence, to estimate the true population proportion with precision-4 and 95 per cent confidence level, the required sample size was 180.

A qualitative immune-chromatographic card test (Sensit Rapid COVID-19 IgG/IgM, UBIO Biotech, Cochin) was done to detect COVID-19 IgG antibodies in all vaccine beneficiaries before vaccination to serve as a benchmark for the comparison of results after vaccination. Those who contracted COVID-19 after the first dose of the vaccine, were excluded from the study. Staff with previous documented COVID-19 infection either by reverse-transcription polymerase chain reaction or by antigen test or positive on IgG card test were also included in the study (if they were in the list generated by random selection) to evaluate any difference in antibody response to vaccination among those with previous COVID-19 infection.

Vaccine beneficiaries were contacted after 28 days of receiving their first dose and between 28-35 days after their second dose of ChAdOx1 nCoV-19 vaccine for blood collection. Seroconversion was measured at 28 days as is on the package insert of ChAdOx1 nCoV-19 vaccine[5,6]. Both doses of vaccine were taken within a gap of 28-42 days. Antibody test was performed using the VITROS® Anti-SARS-CoV-2 IgG (Ortho-Clinical Diagnostics, Rochester, NY, USA) a test for the detection of serum IgG antibodies to the immune-dominant S1 spike of the virus protein of SARS-CoV-2, having a sensitivity of >90 per cent and specificity of 100 per cent[7]. The VITROS anti-SARS-CoV-2 IgG test was approved for use under the Food and Drug Administration's Emergency Use Authorization. Seroconversion was defined as having detectable anti-SARS-CoV-2 IgG antibodies ≥1 on the test. Interpretation of results was as follows: a signal-to-cut-off ratio (S/Co) ≥1 was indicative of a positive result indicating seroconversion. A value <1 was negative for seroconversion. Antibody titre 12 or greater was considered as a high titre while measuring for anti-SARS-CoV-2 antibodies[8,9].

Data collection was done in the vaccination area itself, when the beneficiaries came for their immunization by four clinical pharmacy specialists. They were recruited for data collection after a training session and a quality check was done by the principal investigator for five per cent of the samples. Data included socio-demographic details, antibody titre value after the first and second doses of vaccine, details about comorbidities, the habit of exercising (more than 30 min for at least three days in a week), presence of any perceived stress or decreased sleep (less than six hours of sleep for more than three days in a week).

Statistical analysis was performed using the SPSS software v.21 (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY, USA: IBM Corp.). The categorical variables were expressed as percentages. Chi-square was used to test for associations. Mean antibody titres across different groups were compared using a t test. Logistic regression analysis was also done. Box and Whisker plot was used to display the variation in antibody titres across different groups.

Results & Discussion

The study was conducted on 200 vaccine beneficiaries 28 days after the first dose and between 28 and 42 days after receiving the second dose of the vaccine. Majority of the vaccine recipients belonged to the age group of 30-50 years. Their mean age was 39.94±11.24 yr (minimum-24 yr, maximum-75 yr). One hundred and twenty two (61%) of the vaccine recipients were females. Previous documented COVID-19 infection was present in 10.5 per cent (n= 21/200) of the vaccine beneficiaries. Twenty one were positive on the immunochromatographic card test for COVID -19 IgG antibodies.. All these people had a previous history of documented COVID-19 infection. A total of 19 per cent had either hypertension (HTN) or diabetes and 27 per cent were on treatment for chronic diseases.

Seroconversion after the second dose of the vaccine was seen in 99.5 per cent (199/200) of the beneficiaries as compared to only 91.5 per cent (183/200) after the first dose of the vaccine. After excluding those with previous COVID-19 infection (n=21), the second dose attained seroconversion in 99.4 per cent (178/179) and the first dose attained seroconversion in 90.5 per cent (162/179) of the vaccine beneficiaries. The mean titre after the second dose was significantly (P<0.001) higher when compared to the first dose (12.68±4.17 *vs*. 9.83±6.3). There was an antibody titre ≥12 in 54 per cent (n=108) of the vaccine recipients. Antibody titres did not have any significant association with age, gender, co-morbidities (HTN and diabetes) or factors such as exercise, sleep or stress (Table I). The single most important factor that was associated with high antibody titre was prior infection with SARS CoV-2 (P=0.026).

Participants with prior COVID-19 infection were detected to have higher immunogenicity in comparison to SARS-CoV-2 naïve participants. Antibody titre after the first dose in those without previous COVID-19 (n=179) was 8.69±7.43 and with previous COVID-19 infection (n=21) was 20.5±3.7. Antibody titre after the second dose in those without previous COVID-19 was 12.8±3.7 and with previous COVID-19 was 17.3±2.8. Those with previous COVID-19 infection and only one vaccine shot had a titre [mean-17.81±5.94, median-20.5 (IQR-3.7)] significantly higher than that found in SARS CoV-2 naïve individuals even after the second dose [mean-12.29±4.00, median-12.8 (IQR-3.7), P=0.001]. However, among those with previous COVID-19 infection, there was a fall in titre after the second dose [mean-16.06±4.12 and median-17.3 (IQR-2.8)] of the vaccine when compared to the titre after the first dose [mean-17.81±5.94, median-20.5 (IQR-3.7)].

A decrease in titre following the second dose of vaccination was seen in 28 per cent (n=56) of the vaccine beneficiaries. The mean fall in titre was 2.25±1.40. Previous COVID-19 infection and gender being a male were significantly associated with a decrease in titre following the second dose of vaccination (Table II). Furthermore, the older age group did not have a fall in titre after the second dose when compared to the younger age group. Among those with previous COVID-19 infection, nearly three-fourths of them had a fall in titre following the second dose (Table II). Despite a reduction in titre after the second dose, it was still significantly (P<0.001) higher when compared to SARS-CoV-2 naïve individuals (16.06±4.02 *vs*. 12.29±4.00). Fall in antibody titre did not have any significant association with co-morbidities such as HTN and diabetes (Table II).

Seroconversion following the second dose of the vaccine was seen in 99.5 per cent of the vaccine recipients. In a study by Eyre *et al*[10], all HCWs assessed 14 days after the second dose were seroconverted. Seroconversion following ChAdOx1 nCoV-19 vaccine was reported to be 91 per cent[11], Sputnik V - 88.9

Table I. Factors influencing antibody titres after the second dose of COVID-19 vaccination

Variables	Antibody titre		Total	OR (CI)	*P*
	≥12, n (%)	<12, n (%)			
Age (yr)					
<45	80 (55.9)	63 (44.1)	143		
45-59	22 (53.7)	19 (46.3)	41	0.503 (0.14-1.78)	0.288
≥60	6 (37.5)	10 (62.5)	16	0.907 (0.42-1.95)	0.803
Gender					
Male	37 (47.4)	41 (52.6)	78	1.869 (0.99-3.51)	0.052
Female	71 (58.2)	51 (41.8)	122		
Diabeties present					
Yes	8 (44.4)	10 (55.6)	18	0.689 (0.23-2.11)	0.514
No	100 (54.9)	82 (45.1)	182		
Hypertension					
Yes	10 (47.6)	11 (52.4)	21	1.250 (0.42-3.69)	0.687
No	98 (54.7)	81 (45.3)	179		
Exercises regularly					
Yes	58 (59.2)	40 (40.8)	98	0.546 (0.29-1.01)	0.056
No	50 (49)	52 (51)	102		
Sleep disturbances					
Present	25 (56.8)	19 (43.2)	44	1.160 (0.55-2.41)	0.691
Absent	83 (53.2)	73 (46.8)	156		
Stressed up					
Yes	40 (58.8)	28 (41.2)	68	0.766 (0.41-1.41)	0.397
No	68 (51.5)	64 (48.5)	132		
Previous h/o COVID infection					
Yes	16 (76.2)	5 (23.8)	21	3.468 (1.16-10.34)	0.026
No	92 (51.4)	87 (48.6)	179		

CI, confidence interval; OR, odds ratio; h/o, history of

per cent[12] and CoronaVac (Sinovac Life Sciences, Beijing, China) 83.3 per cent in healthy adults[12]. The efficacy after the second dose of the ChAdOx1 nCov-19 vaccine was 70 per cent (55%-81%)[12] and the seroconversion rate was claimed to be 98 per cent on the product package insert[7]. Therefore, there was an incomplete relationship or absence of any linear correlation between seroconversion and protection from COVID-19, as seroconversion rate post-vaccination exceeded the proportional reduction in the incidence of COVID-19. Thus, the best correlate of protection may be a combined measure of cellular and humoral immunity, clinical outcomes, and antibody responses, which should contribute to prioritization decisions. The main determinant of antibody responses after the second dose was a previous COVID-19 infection. This was also true for studies conducted with mRNA vaccines (*i.e.* after a single dose of the Pfizer-BioNTech vaccine), people with a prior COVID-19 infection had antibody levels similar to those who had taken two doses and without prior COVID-19 infection[13]. Khoury *et al*[14] reported a decline in neutralization titre eight months after SARS-CoV-2 infection.

Among those with previous COVID-19 infection, the majority (71.4%) had a fall in titre after the second dose of vaccination. This could be due to the possible synergistic effect of the anti-spike IgG antibodies after natural infection and the vaccine, leading to initial high antibody titres (not solely due to vaccination) and the natural antibodies waning over time.

Despite not having a previous COVID infection, 23 per cent of the vaccine recipients had a fall in titre after the second dose of the vaccine. This may be a

Table II. Factors associated with decreased titre following vaccination

Variables	Decreased titre		Total	OR (CI)	*P*
	Yes, n (%)	No, n (%)			
Age (yr)					
<45	47 (32.9)	96 (67.1)	143		
45-59	5 (12.2)	36 (87.8)	41	0.912 (0.18-4.52)	0.910
≥60	4 (25)	12 (75)	16	0.279 (0.09-0.86)	0.026
Gender					
Male	17 (21.8)	61 (78.2)	78	2.738 (1.20-6.21)	0.016
Female	39 (32)	83 (68)	122		
Diabeties present					
Yes	2 (11.1)	16 (88.9)	18	0.321 (0.05-1.79)	0.195
No	54 (29.7)	128 (70.3)	182		
Hypertension					
Yes	5 (23.8)	16 (76.2)	21	2.305 (0.58-9.03)	0.231
No	51 (28.5)	128 (71.5)	179		
Previous h/o COVID infection					
Yes	15 (71.4)	6 (28.6)	21	15.955 (4.77-53.32)	0.001
No	41 (22.9)	138 (77.1)	179		

CI, confidence interval; OR, odds ratio; h/o, history of

matter of concern with the vaccines using adenoviral vectors. One suggested possibility is the formation of anti-adenoviral antibodies after the first dose of the vaccine, which could have nullified the effect of the second dose due to an immune reaction to the vaccine vector itself. This would have led to a reduction in anti-spike antibodies after the second dose of vaccination[15]. Although older people, males and those with long-term health conditions were commonly low responders[16], it was noted that patient characteristics such as age and gender and co-morbid conditions such as diabetes did not have any significant impact on the antibody titres after the second dose of vaccine in our population.

Long-term vaccine efficacy requires the persistence of vaccine antibodies above protective thresholds and/or the maintenance of immune memory cells that could rapidly and effectively reactivate with subsequent microbial exposure. Although some studies support that SARS-CoV-2 antibody levels declined with time, others have shown that immune cells can also provide protection from the virus over time[12]. Repeated doses of virus-based vaccines tend to be increasingly less effective because of an immune response mounted against the adenovirus. Mixing and matching vaccines could be another viable, more potent option[4].

Limitations of the study were the lack of evidence of cell-mediated immunity in establishing vaccine efficacy and enrolling only HCWs, who were not the representative of the larger population. Additional research is needed to understand about fall in antibody titres after the second dose and also the duration of protection provided by humoral immunity.

In conclusion, the study suggests that seroconversion after the two doses of the COVID-19 vaccine was 99.5 per cent. Greater immunogenicity was seen among those with previous COVID-19 infection, with a mean antibody titre higher than that found even after the second dose in SARS-CoV-2 naïve vaccine recipients. A fall in antibody titre following the second dose was observed in 28 per cent of vaccine recipients. Except for the presence of a previous COVID-19 infection, antibody titres following the second dose did not have any association with age, gender or co-morbidities in the vaccine recipients.

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***Conflicts of Interest*:** None.

References

1. World Health Organization. *AstraZeneca ChAdOx1-S/nCoV-19 [recombinant], COVID-19 vaccine*. Available from: *https://www.who.int/publications/m/item/chadox1-s-recombinant-covid-19-vaccin e*, accessed on June 11, 2021.
2. Earle KA, Ambrosino DM, Fiore-Gartland A, Goldblatt D, Gilbert PB, Siber GR, *et al.* Evidence for antibody as a protective correlate for COVID-19 vaccines. *Vaccine* 2021; *39* : 4423-8.
3. Heffernan JM, Keeling MJ. Implications of vaccination and waning immunity. *Proc Biol Sci* 2009; *276* : 2071-80.
4. Callaway E. Mix-and-match COVID vaccines trigger potent immune response. *Nature* 2021; *593* : 491.
5. Ramasamy MN, Minassian AM, Ewer KJ, Flaxman AL, Folegatti PM, Owens DR, *et al.* Safety and immunogenicity of ChAdOx1 nCoV-19 vaccine administered in a prime-boost regimen in young and old adults (COV002): A single-blind, randomised, controlled, phase 2/3 trial. *Lancet* 2021; *396* : 1979-93.
6. Serum Institute of India. *ChAdOx1 nCoV- 19 corona virus vaccine (Recombinant)*. Available from: *https://www.seruminstitute.com/pdf/covishield_ChAdOx1_nCoV19_corona_virus_vaccine_insert.pdf*, accessed on January 18, 2022.
7. VITROS Immunodiagnostic. *Instructions for use CoV2G*. Available from: *https://www.mycts.org/Portals/0/COVID19/VitrosIgG.pdf,* accessed on June 13, 2021.
8. Joyner MJ, Senefeld JW, Klassen SA, Mills JR, Johnson PW, Theel ES, *et al.* Effect of convalescent plasma on mortality among hospitalized patients with COVID-19: Initial three-month experience. *medRxiv* 2020. Doi: 10.1101/2020.08.12.20169359.
9. Rojas M, Rodríguez Y, Monsalve DM, Acosta-Ampudia Y, Camacho B, Gallo JE, *et al.* Convalescent plasma in Covid-19: Possible mechanisms of action. *Autoimmun Rev* 2020; *19* : 102554.
10. Eyre DW, Lumley SF, Wei J, Cox S, James T, Justice A, *et al*. Quantitative SARS-CoV-2 anti-spike responses to Pfizer-BioNTech and Oxford-AstraZeneca vaccines by previous infection status. *Clin Microbiol Infect* 2021; *27* : 1516.e7-14..
11. Zhang Y, Zeng G, Pan H, Li C, Hu Y, Chu K, *et al.* Safety, tolerability, and immunogenicity of an inactivated SARS-CoV-2 vaccine in healthy adults aged 18-59 years: A randomised, double-blind, placebo-controlled, phase 1/2 clinical trial. *Lancet Infect Dis* 2021; *21* : 181-92.
12. Voysey M, Clemens SAC, Madhi SA, Weckx LY, Folegatti PM, Aley PK, *et al.* Safety and efficacy of the ChAdOx1 nCoV-19 vaccine (AZD1222) against SARS-CoV-2: An interim analysis of four randomised controlled trials in Brazil, South Africa, and the UK. *Lancet* 2021; *397* : 99-111.
13. National Institutes of Health. *Immune response to vaccination after COVID-19*. Available from: *https://www.nih.gov/news-events/nih-research-matters/immune-response-vaccination-after-covid-19*, accessed on May 28, 2021.
14. Khoury DS, Cromer D, Reynaldi A, Schlub TE, Wheatley AK, Juno JA, *et al.* Neutralizing antibody levels are highly predictive of immune protection from symptomatic SARS-CoV-2 infection. *Nat Med* 2021; *27* : 1205-11.
15. Zhu FC, Guan XH, Li YH, Huang JY, Jiang T, Hou LH, *et al.* Immunogenicity and safety of a recombinant adenovirus type-5-vectored COVID-19 vaccine in healthy adults aged 18 years or older: A randomised, double-blind, placebo-controlled, phase 2 trial. *Lancet* 2020; *396* : 479-88.
16. Wei J, Stoesser N, Matthews PC, Ayoubkhani D, Studley R, Bell I, *et al.* Antibody responses to SARS-CoV-2 vaccines in 45,965 adults from the general population of the United Kingdom. *Nat Microbiol* 2021; *6* : 1140-9.

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