Original Research Article

Effect of previous infection and vaccination on incidence of Covid-19 among healthcare workers and antibody response

ABSTRACT

Aims: To correlate the effect of previous COVID infection and vaccination on incidence of COVID -19 infection .
Study design: It is a prospective observational study.
Place and Duration of Study: Eternal Heart Care Centre and Research Institute, Jaipur, India
Methodology: We included 405 healthcare workers with COVID-19 positive infection, with or without the symptoms. Clinical as well as hematological examination were done.
Results: Our study showed 75.6% seropositivity rate even 6 months after vaccination in which seropositivity was significantly higher in people who had past history of COVID-19 .antibody levels were highest when observed one month after vaccination. A decrease in antibody levels over time after vaccination is observed. Seropositivity rate was significantly higher in people who were less than 40 years old.
Conclusion: A decrease in antibody levels over time after vaccination is observed and this showed that the rate of infection decreases after receiving vaccination.

1. INTRODUCTION

Coronavirus is an RNA virus that belongs to the Coronaviridae family (order: Nidovirales) that causes severe illness in both people and animals. The most common human coronaviruses are 229E, NL63, OC43, and HKU1. There are seven varieties of coronavirus that have been identified and are responsible for causing illnesses in people around the world. The severe acute respiratory syndrome coronavirus (SARS-CoV), Middle East respiratory syndrome coronavirus (MERS-CoV), and severe acute respiratory syndrome coronavirus 2 have all been found in humans as zoonotic coronaviruses (CoV) (SARS-CoV-2). SARS-CoV-2 is an RNA virus that was discovered for the first time in Wuhan, China, in December 2019.(1)

Inflammatory pneumonia is the result of this virus's impact on human lower respiratory tracts. People who are symptomatic, asymptomatic, or presymptomatic can easily spread these viruses to one other, primarily by respiratory droplets when they are in close proximity to one another. Due to its extensive infectivity pattern, the World Health Organization (WHO) declared the new coronavirus (COVID-19) outbreak a global pandemic on March 11, 2020. With more than 200 million illnesses and more than 4 million fatalities worldwide to far, the

coronavirus disease 2019 (COVID-19) pandemic continues to have an unparalleled impact on global health and the world economy.

The discovery of effective and safe vaccines has elevated to a top priority in every area of global health due to the disastrous COVID-19 pandemic. The immunisation campaign against SARS-CoV-2 was commenced in India on January 13, 2021, and two vaccine types—BBV152 (Covaxin) and ChAdOx1—began to be administered (Covishield). The SII, Oxford University, and AstraZeneca created ChAdOx1 (COVISHIELD), a recombinant, replication-deficient chimpanzee adenovirus vector containing the SARS-CoV-2 Spike (S) glycoprotein (2). Following delivery, a portion of the corona virus' genetic material is expressed, inducing an immunological response.(4)

The host mounts an immunological response to the virus following SARS-CoV-2 infection, which often includes the creation of particular antibodies against viral antigens. IgM and IgG antibodies against SARS-CoV-2 appear to develop in blood almost simultaneously. In COVID-19 patients, levels and timing of antibodies vary significantly between individuals, but median seroconversion has been seen to occur at around 2 weeks. The particular antibody titer can be found by quantifying the antibody response, which can also help with longitudinal monitoring of the dynamics of the antibody response in specific patients(3).

The objective of this study is to evaluate the quantitative change in antibody levels in individual who received two doses of ChAdOx1 (COVISHIELD), and its protection against the SARS-CoV-2 infection in healthcare workers in a hospital in India.

2. MATERIAL AND METHODS

1. STUDY DESIGN AND PARTICIPANTS

405 healthcare workers of Eternal Hospital, Jaipur, Rajasthan , India were recruited for this study, out of which 296 were male and 109 were female. It is an observational study which started from January 16, 2021 and ended on June 30, 2022 with one year follow up of all the recipients enrolled. Each participant completed a survey to provide information, which included their age, sex, history of COVID-19 infection, chronic medical problems, height, and weight. An SARS-CoV2 polymerase chain reaction result that was positive led to the diagnosis of COVID-19. The participants were administered ChAdOx1 (COVISHIELD) vaccine in a dose of 0.5 mL in a two-dose schedule, with the doses given at interval of 4-6 weeks (now revised to 8-12 weeks), intramuscularly in the deltoid. Samples were collected from participants to check antibody responses before vaccination as well as after both the doses of vaccination.

2. MEASUREMENT

The Elecsys Anti-SARS-CoV-2 S assay employs a recombinant protein corresponding to the S antigen's RBD in a double-antigen sandwich assay format, and facilitates the quantitative identification of high-affinity antibodies against SARS-CoV-2. (5)

- In the first incubation, a sandwich complex made up of 12 litres of sample, SARS-CoV-2 S-RBD-specific recombinant antigen that has been biotinylated, and SARS-CoV-2 S-RBD-specific recombinant antigen that has been tagged with a ruthenium complex.
- Second incubation: Through the interaction of biotin and streptavidin, after the addition of microparticles coated in streptavidin, the complex is bonded to the solid phase.
- After being aspirated into the measuring cell with the reaction mixture, the microparticles are magnetically attracted to the electrode's surface. ProCell ll M is then used to eliminate unbound chemicals. A photomultiplier measures the chemiluminescent emission that is caused when a voltage is applied to the electrode.

- Results are ascertained using a master curve supplied through the cobas link and a calibration curve created individually for the device using 2-point calibration.
- **ANTIBODY LEVELS-** The analyzer automatically calculates the analyte concentration of each sample in U/ml. If the result is < 0.80 U/ml then the sample is negative for anti-SARS-CoV-2-S and if the result is ≥ 0.80 U/ml then the sample is positive for anti-SARS-CoV-2-S.

3. STATISTICAL ANALYSIS

The mean, median, standard deviation, and interquartile range, when appropriate, were used to summarize the descriptive data. For data on a continuous scale provided as Median (Interquartile range, IQR), the Kruskal-Wallis H test was used to assess differences between two or more data groups. For related samples, Friedman/W Kendall's test was used to determine the significance of the difference. All analyses were carried out utilizing the SPSS programme (Statistical Package for Social Sciences).

4. ETHICAL PERMISSION

The study started after obtaining permission from the Institutional Ethics Committee of the Eternal Hospital (Unit of Eternal Heart Care Centre and Research Institute), Jaipur, Rajasthan, India and written informed consent was taken from all the participants.

5. RESULTS AND DISCUSSION

5.1 STUDY POPULATION

405 participants were included in this study with mean age 35.4±8.5 out of which 296(73.1%) were males and 109(26.9%) were females with the mean age of 35.5±8.6 and 34.9±8.1 respectively. 321(79.2%) were less than 40 years old and 84(20.7%) were more than 40 years old. They received at least two doses of vaccine and 150 of these received the third dose (Booster dose). Median date of first dose was January 25, 2021, median date of second dose was March 3, 2021 and median date of third dose was February 17, 2022. Data decreases, as the follow up of each participant was not possible due to various reasons.

5.2 INFECTION

5.2.1 COVID before vaccination

87(21.4%) participants were tested with covid-19 before the first dose of vaccine, in which 64(72.4%) were males and 23(27.6%) were females. From these participants having previous infection,01(1.2%) participant was tested with covid-19 after the first dose of vaccine, 07(8.1%) participants were tested with covid-19 after the second dose of vaccine and 01(1.2%) participant was tested with covid-19 after the third dose(Booster dose).

318(78.27%) participants were not tested with covid-19 before the first dose of vaccine, in which 232(73.2%) were males and 86(26.8%) were females. From these participants not having previous infection, 14(4.4%) participants were tested with covid-19 after the first dose of vaccine, 17(5.4%) participants were tested with covid-19 after the second dose of vaccine and 3(0.95%) participants were tested with covid-19 after the third dose (Booster dose).

5.2.2 COVID after vaccination

18(4.4%) participants were tested with covid-19 after the first dose of vaccine, in which most were males (61.1%), 28(6.9%) participants were tested with covid-19 after the second dose

of vaccine, in which most were males (75%) and 04(21.4%) participants were tested with covid-19 after the third dose (Booster dose), in which all were males.

Table 1-Distribution of study group on basis of incidence of infection .

	Infection	**No Infection**
Before vaccination (n=405)	**87(21.5%)**	**246(60.7%)**
Post-vaccination		
After 1st Dose (n=405)	**18(4.4%)**	**386(95.3%)**
Vaccination and Past Covid-19 (n=87)	**1(1.1%)**	**86(98.9%)**
Vaccination and No history of Covid-19 (n=246)	**14(5.7%)**	**232(94.3%)**
After 2nd Dose (n=208)	**28(13.5%)**	**180(86.5%)**
Vaccination and Past Covid-19 (n=87)	**7(8.0%)**	**41(47.1%)**
Vaccination and No history of Covid-19 (n=246)	**17(6.9%)**	**125(50.8%)**
After 3rd Dose (n=208)	**4(1.9%)**	**204(98.1%)**
Vaccination and Past Covid-19 (n=87)	**1(1.1%)**	**47(54.0%)**
Vaccination and No history of Covid-19 (n=246)	**3(1.2%)**	**139(56.5%)**

5.3 ANTIBODY LEVELS

5.3.1 Before vaccination

Evaluation of antibody levels were conducted for each participant before the first dose of vaccine was given. It showed 36.3% seropositivity, with a median antibody level of 0.11(0.06-4.12) U/ml. Where people who had previous history of covid-19, showed 59% seropositivity with a median antibody level of 2.20(0.10-15.0) U/ml and people who didn't, showed 29.5% seropositivity with a median antibody level of 0.08(0.06-1.73) U/ml.

5.3.2 1 Month after vaccination

One month after the second vaccine dose, blood samples were obtained from 280 participants. There was a 30.8% loss in the number of participants compared to the previous blood draw. Antibody levels were evaluated and it showed 62.5% seropositivity, with a median antibody level of 4.91(0.09-32.2) U/ml. Seropositivity rate was significantly higher in participants who had previous history of covid-19(86.9%) with a median antibody level of 19.7(4.1-93.9) U/ml then in people who didn't (56.6%) with a median antibody level of 2.29(0.09-21.5) U/ml. Whereas 105(37.5%) participants were still seronegative. Seropositivity rate was significantly higher in people who were less than 40 years old.

Highest antibody levels were found to be 260 U/ml. 266 participants were found to be covid-1 9 protected and 13 were found covid-19 infected at 1 month after vaccination, with median antibody level of 6.00(0.09-33.1) U/ml and 0.090(0.068-0.093) U/ml respectively.

5.3.3 6 Months after vaccination

Six months after the second vaccine dose, blood samples were obtained from 132 participants. There was a 52.8% loss in the number of participants compared to the previous blood draw. Antibody levels were evaluated and it showed 75.6% seropositivity, with a median antibody level of 4.07(0.83-29.6) U/ml. Seropositivity rate was significantly higher in participants who had previous history of covid-19(87.9%) with a median antibody level of 5.4(2.0-48.3) U/ml then in people who didn't (72.5%) with a median antibody level of 3.13(0.43-21.5) U/ml. Whereas 32(24.4%) participants were still seronegative. Seropositivity rate was significantly higher in people who were less than 40 years old. Highest antibody levels were found to be 197.3 U/ml. 122 participants were found to be covid-19 protected and 09 were found covid-19 infected at 6 months after vaccination, with median antibody level of 3.30(0.74-23.2) U/ml and 24.4(8.92-75.4) U/ml respectively.

Table 2- Distribution of study group on basis of antibody levels .

	Previous Infection	**No Infection**
Ab+		
Before Vaccination(n=132)	**49(37.1%)**	**67(50.8%)**
After 1 Month(n=175)	**53(30.3%)**	**90(51.4%)**
After 6 Months(n=99)	**29(29.3%)**	**58(58.6%)**
Ab-		
Before Vaccination(n=232)	**34(14.7%)**	**160(69%)**
After 1 month(n=105)	**8(7.6%)**	**69(65.7%)**
After 6 Months(n=32)	**4(12.5%)**	**22(68.8%)**

Fig 1- Overall antibody levels (n=405) Antibody levels in people who had symptomatic as well as asymptomatic Covid-19 Infection

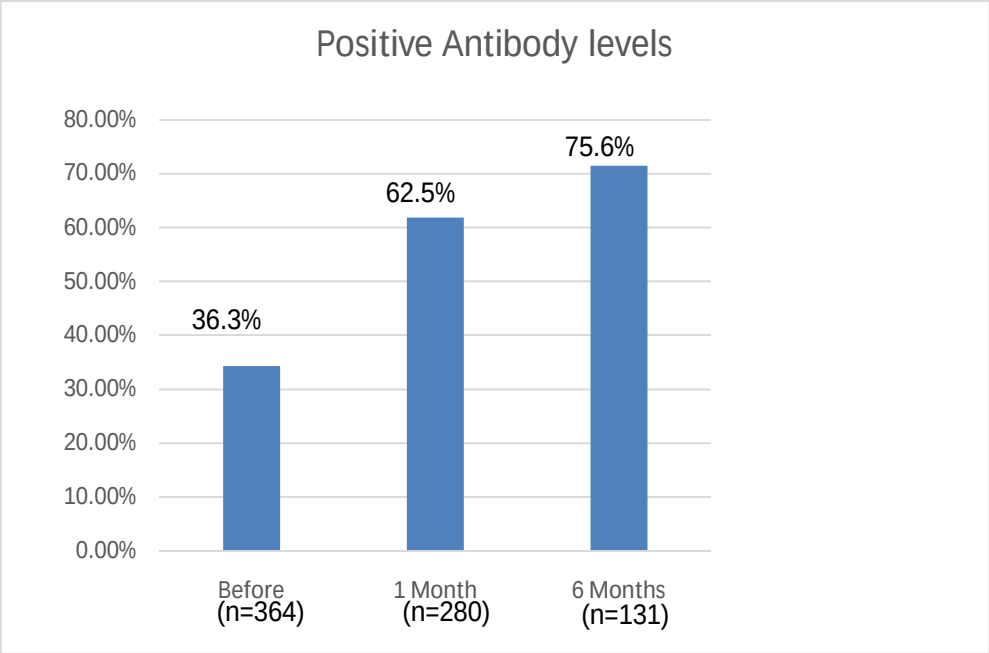

Fig 2-Antibody levels in people who had symptomatic Covid-19 Infection before vaccination (n=87)

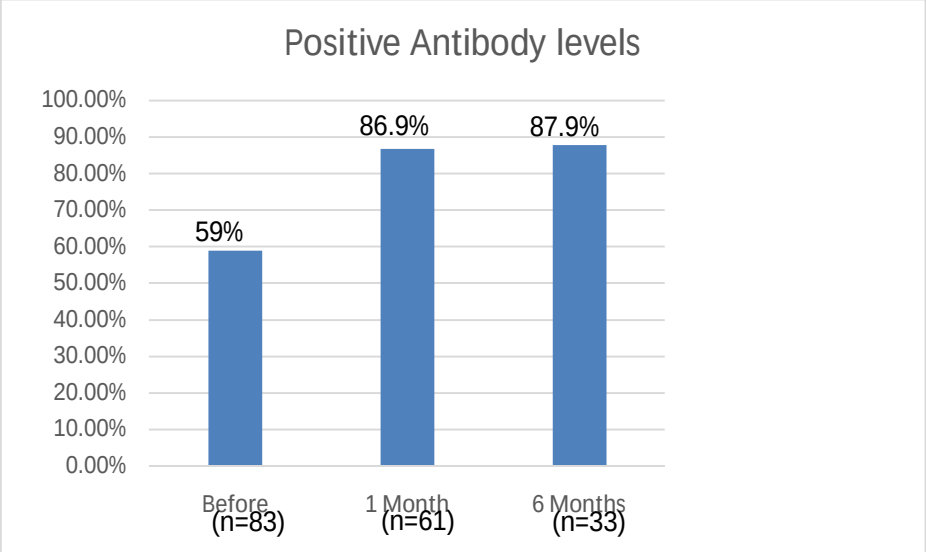

Fig 3- Antibody levels before & after vaccination without history of Covid-19 Infection (n=246)

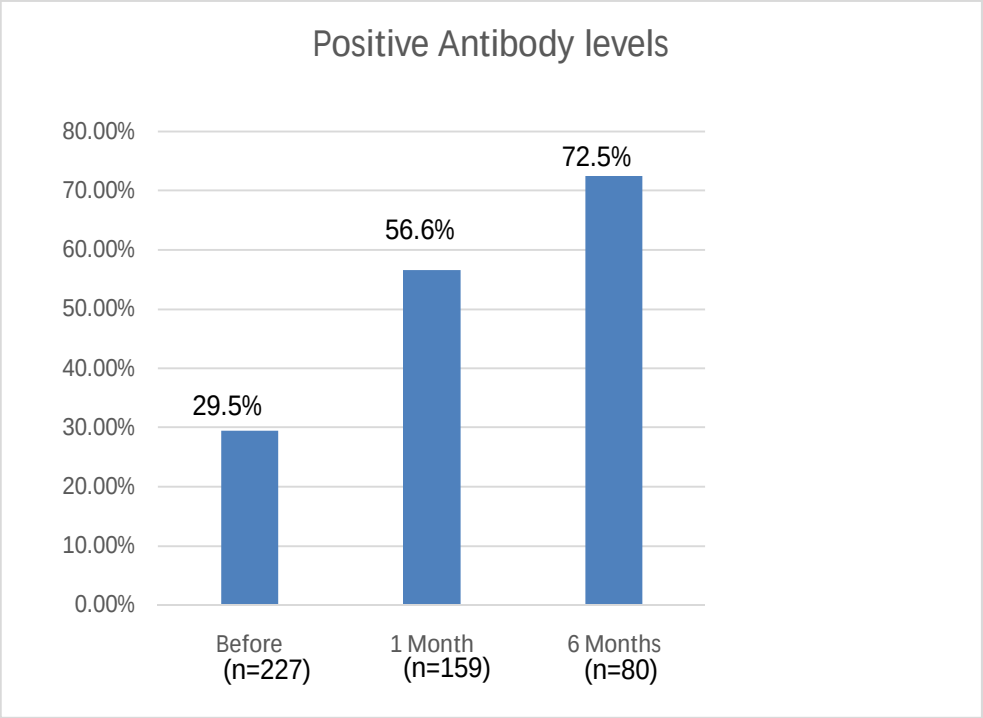

Table 3- Trends in nucleocapsid antibody levels (mg/dl) following 2-dose vaccination in various groups

Total cohort (n=405)	**Baseline. Before vaccination**	**1 months after vaccination**	**6 months after vaccination**	**P_{trend}** **ANOVA/ Kruskal-Wallis)**
Number	(N=368)	(N=280)	(N=132)	
Mean (SD) Median (IQR)	8.3±22.9 0.11(0.06-4.12)	29.8±51.2 4.91(0.09-32.2)	23.2±38.4 4.07(0.83-29.6)	<0001 <0.001
Pre-vaccination status	**(N=280)**	**(N=218)**	**(N=97)**	
No past Covid-19 infection Mean (SD) Median (IQR)	 5.5±16.6 0.08(0.06-1.73)	 24.2±46.2 2.29(0.09-21.5)	 20.7±37.1 3.13(0.43-21.5)	 <0.001 <0.001

	(N=87)	**(N=61)**	**(N=35)**	
Previous Covid-19 infection (n=87) Mean (SD) Median (IQR)	 17.3±35.1 2.20(0.10-15.0)	 49.9±62.9 19.7(4.1-93.9)	 30.0±41.8 5.4(2.0-48.3)	 0.001 <0.001
Post-vaccination status	**(N=355)**	**(N=266)**	**(N=122)**	
Covid-19 protected (n=355) Mean (SD) Median (IQR)	 8.5±23.1 0.11(0.06-4.54)	 30.7±51.5 6.00(0.09-33.1)	 21.7±37.3 3.30(0.74-23.2)	 <0.001 <0.001
	(N=267)	**(N=204)**	**(N=87)**	
Vaccination and No Covid-19 (n=) Mean (SD) Median (IQR)	 5.6±16.7 0.08(0.06-2.15)	 25.0±46.5 3.11(0.09-22.0)	 18.3±35.0 2.61(0.36-17.4)	 <0.001 <0.001
Vaccination and Past Covid-19 (n=) Mean (SD) Median (IQR)	-	-	-	
	(N=12)	**(N=13)**	**(N=09)**	
Covid-19 infected (n=12) Mean (SD) Median (IQR)	 4.6±15.8 0.061(0.059-0.065)	 13.8±42.6 0.090(0.068-0.093)	 45.9±50.2 24.4(8.92-75.4)	 0.053 0.002

5.4 DISCUSSION

Our study showed 75.6% seropositivity rate, in which seropositivity was significantly higher in people who had past history of covid-19 even 6 months after vaccination. Antibody levels were highest when observed one month after vaccination but seropositivity was observed in

only some cases after 6 months. A decrease in antibody levels over time after vaccination is observed. This study also showed that the rate of infection decreases after receiving vaccination. 87 participants had a past history of covid-19, 28 participants were tested with covid-19 after two doses of vaccination and only 4 participants after the third dose (Booster Dose).

In the 2020 study, Merryn Voysey assessed the safety and efficacy of the ChAdOx1 nCoV-19 vaccine in a pooled interim analysis of four trials. She found that vaccine efficacy was 62.1% in participants who received two standard doses, 90.0% in participants who received a low dose followed by a standard dose, and 70.4% overall in both groups.(5)

In the study from 2021, Awadhesh Kumar Singh assessed the humoral antibody response following the first and second doses of the SARS-CoV-2 vaccines Covishield and Covaxin in Indian healthcare professionals. 21–36 days after the second finished dosage, the combined findings of the two vaccines indicated 95% seropositivity to anti-spike antibody. In the propensity-matched analysis of SARS-CoV-2 naive subjects, seropositivity rates were greater in Covishield recipients than in Covaxin recipients.(6)

Our study does have certain limitations. First, it's possible that the participants with asymptomatic illnesses were inadvertently added to the study group. Second, because they weren't routinely checked by PCR, research participants who had asymptomatic infections might not have been discovered. The third is the potential for selection bias since individuals may have been overlooked for follow-up. At the second and third blood draws, a decline in participants was seen. The main cause might be the pandemic's excessive workload, together with the fact that certain medical staff members worked shifts in our hospital and some even left.

4. CONCLUSION

Over the time vaccination proves to be effective in covid infected patients as a decrease in antibody levels was observed and the rate of covid infection was decreased after vaccination. Our data showed an effective decrease in infection after vaccination and suggests that vaccination drive in countries worldwide must be given a pace.

CONSENT (WHERE EVER APPLICABLE)

"All authors declare that 'written informed consent was obtained from the patient (or other approved parties) for publication of this case report and accompanying images. A copy of the written consent is available for review by the Editorial office/Chief Editor/Editorial Board members of this journal."

ETHICAL APPROVAL (WHERE EVER APPLICABLE)

"All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki."

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